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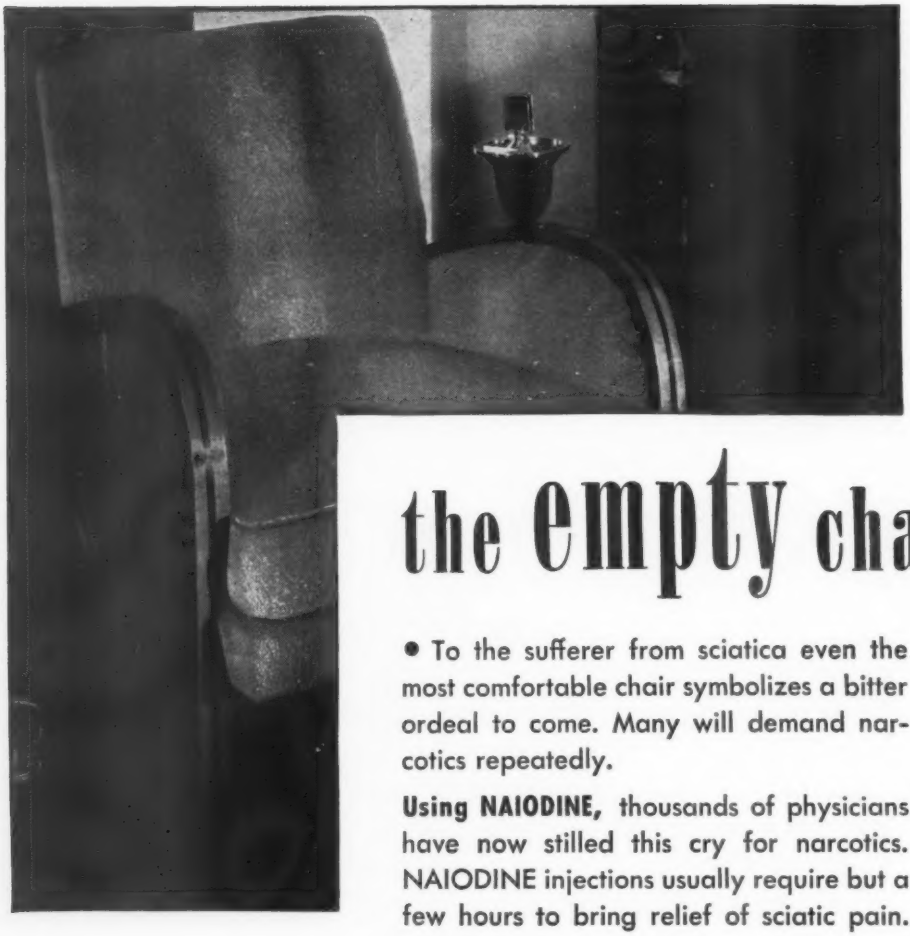

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THE AMERICAN JOURNAL
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SEPTEMBER, 1946

ORIGINAL ARTICLES

FOLIC ACID (SYNTHETIC L. CASEI FACTOR), AN ESSENTIAL PANHEMATOPOIETIC STIMULUS

EXPERIMENTAL AND CLINICAL STUDIES*

BY CHARLES A. DOAN, M.D.

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COLUMBUS, OHIO

THE current era of chemotherapeutic and antibiotic therapeutic triumphs in the field of the infectious diseases has tended to subordinate and overshadow the rôle of optimum nutrition and the importance of an effective mobilization of cellular and humoral immune forces in the resistance economy of the human body. In a world of increasing scientific complexity, with its multiplicity of geographic, social, economic and dietary maladjustments, and with an imminent inevitable upsurge of intercontinental, interracial admixtures of inherited and acquired traits and diseases, it is mandatory to reëvaluate *all* of the factors necessary for maintaining strong bodies and sane mentalities. No longer optional, or to be casually accepted or hoped for, these attributes have become vital to human understanding and happiness, essential to human survival.

It is well within the memory of most of us when really serious and scientific attempts were first made to understand and control the minutia of detail about essential nutritional requirements for optimum growth, development and function—and that progress has been made in the application of this knowledge to the solution of specific human disease problems. Biologic and physical chemistry, and the experimental method, are making increasingly possible both direct and indirect controls over the phenomena of health and disease to an extent previously unimagined and unpredictable.

The interplay of bacterial and viral agents among a population comprising individuals with idiosyncratic and variable physical reactions and responses, dependent upon both environmental and inherited or acquired organic characteristics, has proven all too frequently to be disastrous to persons, to nations and to races. Gradually but surely, at least the physical basis for disease prevention and cure is being laid, and the emphasis, therefore, is steadily shifting toward the psychologic, the emotional aspects of human character—for better or for worse, as was emphasized in last year's Jarecki Lecture. It is the more recently acquired knowledge relating to specific molecular nutrition and to the closely related mammalian cellular mechanism of resistance which has been

* The 12th Edwin A. Jarecki Memorial Lecture, Philadelphia, May 17, 1946.

chosen for presentation in this Memorial Lecture.

It is no mere coincidence that our own first studies of living blood and bone marrow cells were initiated in Baltimore some 25 years ago from the standpoint of nutritional control factors. Prior to Dr. Florence R. Sabin's pioneer studies of embryologic blood formation in the living chick blastoderm,[37] practically all of the recorded observations had been on fixed and stained preparations of sectioned material, and the only studies on simplified hypocellular adult marrow had been from tissues subjected to severely toxic, destructive influences.[44] In an adjacent laboratory, McCollum was in the midst of his synthetic dietary studies in rats with special reference to vitamin factors, and we were permitted and invited to observe the influence of various dietary combinations on the hematopoietic equilibrium. But the material was too complex for satisfactory analysis at that time, since no one of the diets studied provided an appreciable marrow hypoplasia in the rat, and the fundamental underlying cellular structure of this organ, under normal adult conditions, was not yet revealed. It became evident early in our work that an easily induced, reversible, non-toxic, hypoplasia of adult marrow must be available for the initial basic study of bone marrow structure and function.[17] An adult avian form was chosen, the pigeon, in order to correlate more directly Sabin's analysis of erythrogenesis in the chick blastoderm. Simple withholding of all solid food for a relatively brief period was found to produce a progressive panmarrow hypoplasia for all normal elements in the adult pigeon, so that with the resumption of a normal grain diet the prompt but progressive return to normal blood cell formation permitted the observer to note the origins and relationships of the various cell types.[18] Studies followed, in the mammal, as well as the avian species, in the attempt to discover the dietary factors essential for each blood cell type. While Whipple and his associates were establishing the essential dietary constituents for hemoglobinogenesis in anemic dogs, the rôle of the nucleotides in granulocytopoiesis in pigeons and rabbits,[19] and subsequently in human granulopenic states,[20] was being explored.

During the *interim* between World War I and World War II, epochal progress was achieved through the combined genius of Whipple, Minot and Castle, not alone through the specific application of their discoveries to the successful treatment of pernicious anemia but, even more, through the establishment of the principle of nutritional deficiency, on either an intrinsic or extrinsic basis, as solely responsible for the cellular maturation arrest, which tissue pathologists had previously interpreted as malignant megaloblastic anaplasia. This revelation has provided a pattern for the approach to many other unsolved medical problems.

Despite the unavoidable diverting of investigational activities to direct war objectives in the immediate past, nevertheless efforts have been gradually intensified, utilizing modern chemical, physical and pharmacologic techniques, to fractionate, isolate, identify and synthesize specific molecules with specific biologic activity, from vitamin complexes and from many other sources. Slowly but surely biologic investigators are separating the active from the inactive, the specific from the non-specific, the innocuous from the dangerous, the stimulatory from the depressant or inhibitory chemical combines which affect living protoplasm. It is down this avenue of diversified investigations, from many individuals and groups with multiple interests and varied objectives, that has come the information, which is focusing attention sharply at the present time on the so-called folic acid or "*L. casei* factor" concentrates in terms of human bone marrow activity.

It was inevitable that some confusion should arise temporarily concerning this newest subdivision of the rapidly growing vitamin B family, due to its wide distribution in nature, its multiple, species-bio-

logic activities, and its variously acquired nomenclature, derived from widely separated groups of investigators. There are 5 designations, which have been commonly and somewhat indiscriminately applied to this group of compounds: vitamin M, vitamin B_c, eluate factor, folic acid and *L. casei* factor. Briefly, their historical derivations may be indicated. In 1935, Day, Langston and associates published the first of a series of communications,[13,14,15,16,27] describing the development of a nutritional cytopenia in *monkeys* on a vitamin B deficient diet, which could be prevented or corrected by *either* liver extract *or* brewer's yeast. The essential but unidentified component or components common to these 2 foods was designated in 1938 as "vitamin M."[27] Similar studies were started in our laboratory in 1940 in association with Drs. Henry E. Wilson, Jr., Oram Woolpert and Samuel Saslaw, in an attempt to identify the essential hematopoietic factor—more specifically a *granulopoietic* factor—among the growing group of subfractions purified and isolated from the vitamin B complex. Niacin amide, thiamin, riboflavin, calcium pantothenate, pyrodoxin, inosital, para-aminobenzoic acid, choline, pimelic acid and glutamine were used as they became available, and individually and collectively they failed to prevent the gradual development of a pan-marrow hypoplasia in the *Macacus mulatta*, when placed on a restricted diet (Figs. 1 and 2). We concluded[39,60] that the crystalline folic acid obtained from liver by Stokstad[52] and a highly purified concentrate from yeast[34,35] each "closely simulated the effect of parenteral liver extract, reestablishing also a normal white cell equilibrium" in our monkeys (Fig. 1). The identity of vitamin M with these folic acid concentrates was suggested and implied.[39,61] Waisman and Elvehjem[56] confirmed the antileukopenic effect of a "folic acid"-rich norite eluate concentrate for monkeys in 1943, and Day *et al.*[12] reported in 1945 the successful treatment of "vitamin M deficiency" in the monkey with highly purified *L. casei* factor, thus establishing the biologic identity of these 2 factors.

In 1939, Hogan and Parrott[22] described an anemia in chicks due to an undetermined dietary deficiency, which they designated "vitamin B_c," the "c" being appended to identify the chick species. Its isolation from *liver* in crystalline form was accomplished in 1943 by Pfiffner and co-workers,[33] who stated that "it appears probable that the chick anti-anemia factor B_c, Peterson's eluate factor, and William's 'folic acid' are the same." In 1945 these same investigators[34] isolated a second crystalline compound with anti-anemic activity for the chick, this time from *yeast*. The molecular weight, however, was found to be 2.8 times that of vitamin B_c from liver, and it was termed "vitamin B_c conjugate." Typical vitamin B_c was obtained from the "conjugate" on enzyme digestion. Scott *et al.*[43] reported that following severe experimental hemorrhage in hens, the intramuscular injection of *L. casei* factor synthetic or of beta-pyracin hastens the regeneration of hemoglobin, and when both are given together the recovery is still more rapid and a higher total hemoglobin level is attained.

Snell and Peterson[46] in 1940 found that among the requirements for the cultivation of *Lactobacillus casei* was a factor occurring in *yeast, liver* and *other natural materials*, which could be adsorbed on norite and eluted therefrom with ammoniacal alcohol. Stokstad,[53] and Hutchings, Bohonos and Peterson[24] then carried the concentration of this "eluate factor" further, and the latter showed that this concentrate had growth[25] as well as anti-anemic activity[28] for the chick. Similarities in chemical properties between this "norite eluate factor," factor U of Stokstad and Manning,[52] the alcohol precipitate factor of Schumacher *et al.*,[42] and vitamin B_c[22] were pointed out. Then Hutchings, Stokstad, Bohonos and Slobodkin[26] in 1944, using absorption spectra data for identification, grouped all of

these concentrates under the general term "*L. casei* factor."

Mitchell, Snell and Williams[29] reported in 1941 that they had obtained from spinach in relatively pure form an acid nutrilite, which they called "folic acid," and which they found to be essential for the growth of *L. casei* and *Streptococcus lactis*. Many investigators, thereupon, adopted this euphonious designation as a generic term to cover all substances with similar physiologic properties from whatever source derived. In 1944, however, the originators of this cognomen,[30] defined "folic acid" specifically as an essential growth factor for *S. lactis*, thus limiting considerably its strictly literal application to some of the other closely related, but not identical substances.

Daft and Sebrell[7] in their recent review of this subject (1945) adopted and defined the term "*L. casei* factor" as "any substance having activity for the rat and chick, and either active for *L. casei* or *S. lactis*, or capable of being changed to an active form by treatment with acid, alkali, or any enzyme."

Last August, Angier and his associates[1] announced the synthesis of a compound, without revealing its exact structure, which has ultraviolet and infrared absorption spectra, crystalline structure, and biologic activity identical with the *L. casei* factor isolated in pure form from liver by Stokstad.[52] Supplies of the crystalline biologic have been necessarily extremely limited in the past, only $\frac{1}{10}$ ounce of active material resulting from a ton of original fresh liver. The relatively more abundant quantities of folic acid of standardized uniform potency now available has stimulated great interest and investigative activity.

Most of the studies have stressed only the anti-anemia and growth stimulating properties of these concentrates, though the work in monkeys recognized from the beginning leukopenia as well as anemia in the vitamin B deficiency state. A few investigators have noted the possible significance of *L. casei* factor for granulocytopoiesis and thrombocytopoiesis, which would seem an even more important clinical attribute of these molecular complexes, if true.

In a series of studies with rats it was found by Welch *et al.*,[58] Daft and Sebrell *et al.*,[47] and Elvehjem *et al.*[6,36] that agranulocytosis may occasionally occur in the presence of a dietary deficiency only, but the incidence is greatly increased if sulfa drugs, or thiourea and thyroxin[8] are included in the purified diet. Furthermore, crystalline "folic acid"[9] successfully prevents or corrects this development. More recently Daft, Sebrell and associates[10] (1945) have reported severe anemia and/or granulocytopenia, with bone marrow hypoplasia and congestion in a high percentage of rats fed a purified diet low in pantothenic acid, in contrast to their controls. Only 20% of the rats escaped some form or degree of blood dyscrasia. A few ran a very rapid, fulminant fatal course. The therapy consisted of 200 to 5000 mg. daily of pantothenic acid alone or combined with 25 to 100 mg. of *L. casei* factor. They conclude: "the granulocytopenia, when unaccompanied by anemia, appeared in most cases to be the result of *L. casei* factor deficiency, which for reasons unknown, developed more easily in the absence of pantothenic acid. Folic acid alone did not affect the anemia, and pantothenic acid alone did not affect the granulocytopenia. The anemia responded reasonably well to the combined treatment of pantothenic acid and folic acid. It would seem, therefore, at least a possibility that this anemia was due to the simultaneous deficiency of these 2 factors." Along these same lines, Wright and Welch[63] found that feeding rats a highly purified diet containing sulfasuxidine resulted in a marked reduction in the *hepatic stores* of folic acid, biotin and pantothenic acid, the other B group vitamins being unaffected. This occurred despite ordinarily adequate amounts of pantothenic acid *in the diet*, and persisted even after parenteral administration. Crystalline biotin plus folic acid concentrate restored

the pantothenic acid content of the liver to normal with the disappearance of all symptoms. These authors discuss the possibility of folic acid and biotin playing a rôle in the bacterial synthesis of pantothenic acid or in the proper storage or utilization of pantothenic acid in the tissue.

Vitamin B_{10} and B_{11} concentrates[6] proved to be inactive as sources of folic acid for the monkey, while crystalline B_c and synthetic *L. casei* factor were quite adequate. A reversal of the lymphocyte-neutrophil ratio plus sub-optimal hemoglobinogenesis were precipitated by folic acid deficiency.

Finally, Kornberg, Daft and Sebrell[10] have just shown that weanling rats become both granulocytopenic *and* anemic on diets devoid of adequate protein. The simultaneous addition to the diet of both *L. casei* factor and the essential amino acids was necessary for full recovery.

Prior to our own clinical studies with folic acid, extensive experimental investigations were carried out in monkeys, particularly from the standpoint of susceptibility to virus and streptococcus infections in folic acid-deficient, leukopenic states with or without anemia.

Reference to Table 1 will indicate the bone marrow and peripheral cell response to various diets (Table 2) in which the vitamin B complex is lacking in a greater or lesser degree. All monkeys developed a leukopenia after a period of 30 to 120 days on one or other of these diets, and 57% showed an accompanying anemia.[61] In sharp contrast to the nutritionally normal monkey, which is quite resistant to the influenza virus and/or hemolytic streptococcus Group C, the nutritionally leukopenic monkey is extremely susceptible with a high mortality rate (Table 3).

Neutralizing humoral antibodies in re-

TABLE 1.—RED AND WHITE BLOOD CELL RESPONSES OF MACACA MULATTÆ ON MODIFIED VITAMIN B DEFICIENT DIETS

	Total	Developing leukopenia	Developing anemia
Goldberger diet	4	4	3
Synthetic basic diet (vitamin B-free)	4	4	3
Diet I (with supplements)	7	7	4
Diet II (with supplements)	20	20	10
	35	35 (100%)	20 (57%)

TABLE 2.—TWO DIETS LACKING IN VITAMIN B COMPLEX WITH SUPPLEMENTS

Diet No. 1

*Basic Diets**	%	*Vitamin Supplement†*	Daily dosage (mg.)
Sucrose	68	Thiamine hydrochloride	1
Vitamin-free casein	18	Riboflavin	1
Vegetable oil	8	Pyridoxin hydrochloride	1
Salt mixture (U. S. P. No. 2)	4	Nicotinic acid amide	25
Cod liver oil (U.S.P.)	2	Calcium pantothenate	3
	100	Ascorbic acid	25

Diet No. 2

	Daily dosage (mg.)
Diet No. 1 with addition of:	
Choline chloride	50
Pimelic acid	1
Glutamine	1
Inositol	1
Sodium paraminobenzoate	50

* Liberal amounts of the basic diet and water were available to the animals at all times.

† Double doses of supplements suspended in water and fed by stomach tube every 2nd day. Biotin assays were done on feces of some of these animals with and without addition of biotin to the diet. Since these assays indicated synthesis of large amounts in the intestine of the monkey biotin supplements were discontinued.

sponse to the virus,[40] and specific precipitins, opsonins and antistreptolysin titers[41] following the streptococcus developed in time and quantity in the nutritionally deficient monkeys as in the animals on normal diet. The most obvious difference in the 2 groups was the diminished number and inferior quality of the circulating granulocytes, and the progressive hypoplasia of the bone marrow in the absence of folic acid in the diet.

Monkey 61 (Fig. 1) is illustrative of the progressive pan-marrow hypoplasia with

TABLE 3.—SUSCEPTIBILITY TO INFECTION OF MACACA MULATTÆ ON A MODIFIED VITAMIN A DEFICIENT DIET

I. Hemolytic streptococcus (Group C) (intranasal inoculations) 6
Streptococcus septicemia, fatal (7 to 13 days) 5 (83%)

	Diet	Survival	Findings
M 8	600		
M 14	600	8 days	Septicemia
M 23	I	7 days	Sepsis erysipelas
M 55	II	7 days	Septicemia
M 51	II	13 days	Sepsis erysipelas
M 54	II	18 days	Sepsis erysipelas

II. Influenza virus A (Strain PR 8) (intranasal inoculations) 7
Virus infection, fatal (2 to 11 days) 5 (71%)

	Diet	Survival	Pathology	Recovery of virus
M 24	600	3 days	+	+
M 27	I	2 days	+	+
M 51	II			
M 53	II	8 days	+	−
M 54	II			
M 56	II	11 days	+	−
M 69	II	7 days	+	+

Controls on Normal Optimum Diet

III. *Streptococcus hemolyticus* (Group C) (intranasal inoculations) 9
Fatalities 0
Influenza virus A (Strain PR 8) (intranasal inoculations) 10
Fatalities 0
Hemolytic streptococcus followed or preceded by influenza virus 30
Fatal streptococcus septicemia (15 to 21 days) 2 (4%)
(Streptococcus preceded by virus 15 days.)

a resulting peripheral pan-hematopenia which occurs on the unsupplemented basic diet. Immediately, within 24 hours of the administration of a relatively small dosage of folic acid concentrate, the granulocytes were increased in the circulating blood and shortly thereafter both reticulocytes and thrombocytes appeared in greater abundance, followed promptly by a steady and sustained rise in total red blood cells, hemoglobin and lymphocytes. The difference in sequence of these cellular events from those occurring following liver extract (Fig. 2), or when an hyperplastic, megaloblastic, maturation-arrest bone marrow is subjected to folic acid (Fig. 7) is significant. In the latter instance the peripheral leukopenia and thrombocytopenia are usually primarily myelophthisic in mechanism and the reticulocytosis precedes the granulocyte and platelet responses. In the nutritionally hypoplastic marrow in our monkeys, apparently the deficiency for granulocytopoiesis, when corrected, resulted in a reactivation which released mature neutrophils directly and before the red blood cells have had time to respond. The reticulocytosis was greater but the granulocyte response was more delayed and less pronounced in Monkey 136 (Fig. 2) following liver extract, than when folic acid concentrate was administered.

Inasmuch as our early studies[19] on the mechanism of nucleinate induced granulocytosis gave a similar prompt release of white blood cells to the circulation, when

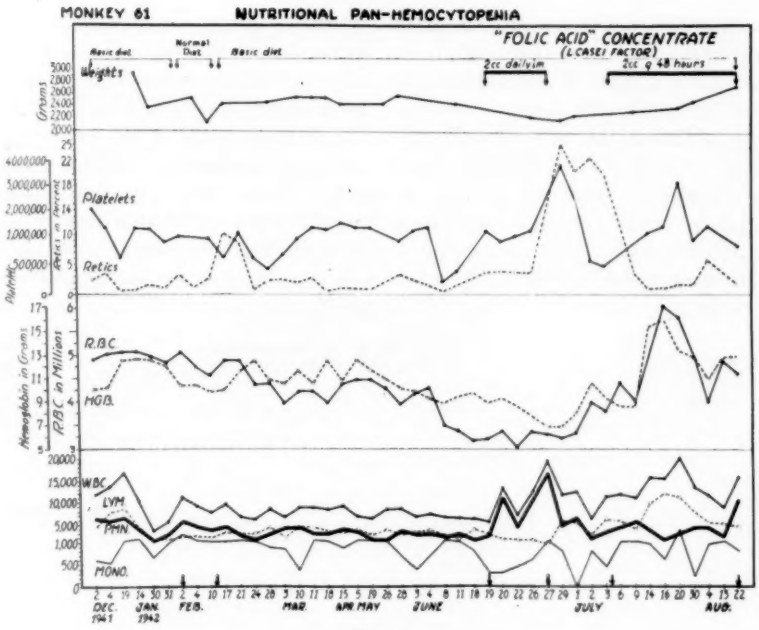
MONKEY 61
NUTRITIONAL PAN-HEMOCYTOPENIA
"FOLIC ACID" CONCENTRATE
(L CASEI FACTOR)
Basic diet
Normal Diet
Basic diet
2cc daily i.m.
2cc q 48 hours
Weights
Grams
Platelets
Retics
Platelets in Millions
Retics in Percent
R.B.C.
HGB.
Hemoglobin in Grams
RBC in Millions
WBC
LYM.
PMN.
MONO.
DEC. 1941
JAN. 1942
FEB.
MAR.
APR MAY
JUNE
JULY
AUG.

FIG. 1.

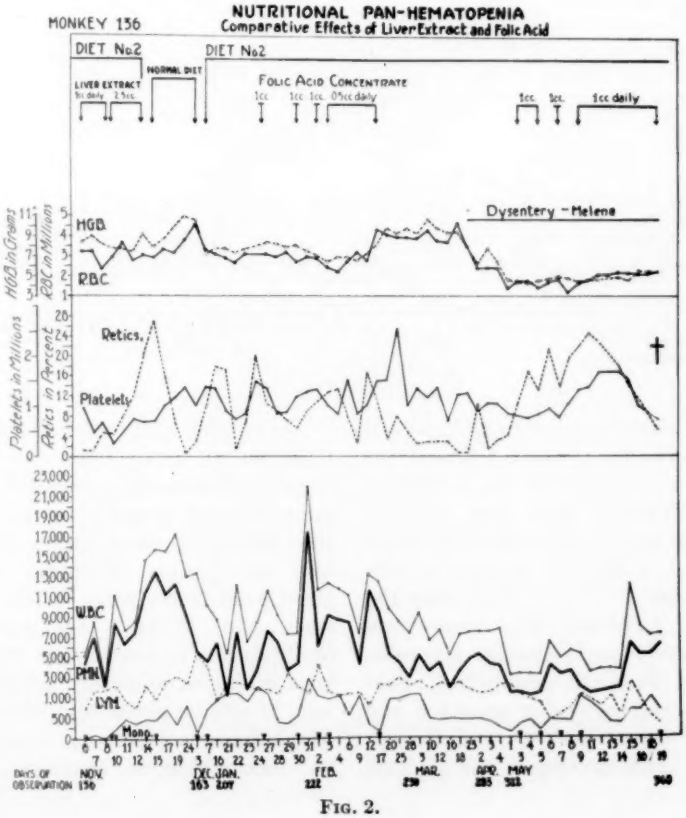
MONKEY 136
NUTRITIONAL PAN-HEMATOPENIA
Comparative Effects of Liver Extract and Folic Acid
DIET No2
DIET No2
LIVER EXTRACT
NORMAL DIET
FOLIC ACID CONCENTRATE
1cc daily
Dysentery - Melena
HGB.
RBC.
HGB in Grams
RBC in Millions
Retics.
Platelets
Platelets in Millions
Retics in Percent
WBC
PMN.
LYM.
Mono.
DAYS OF OBSERVATION
NOV.
DEC. JAN.
FEB.
MAR.
APR. MAY

FIG. 2.

the bone marrow was functionally capable of responding, we were interested in comparing the reaction of our leukopenic monkeys to adenylic acid *versus* folic acid. While the granulocytes rose transitorily in Monkey 1 C (Fig. 3) following each injection of adenylic acid, there was no evidence of a progressive and sustained recovery of both leukocytes and erythrocytes such as occurred on daily folic acid administration.

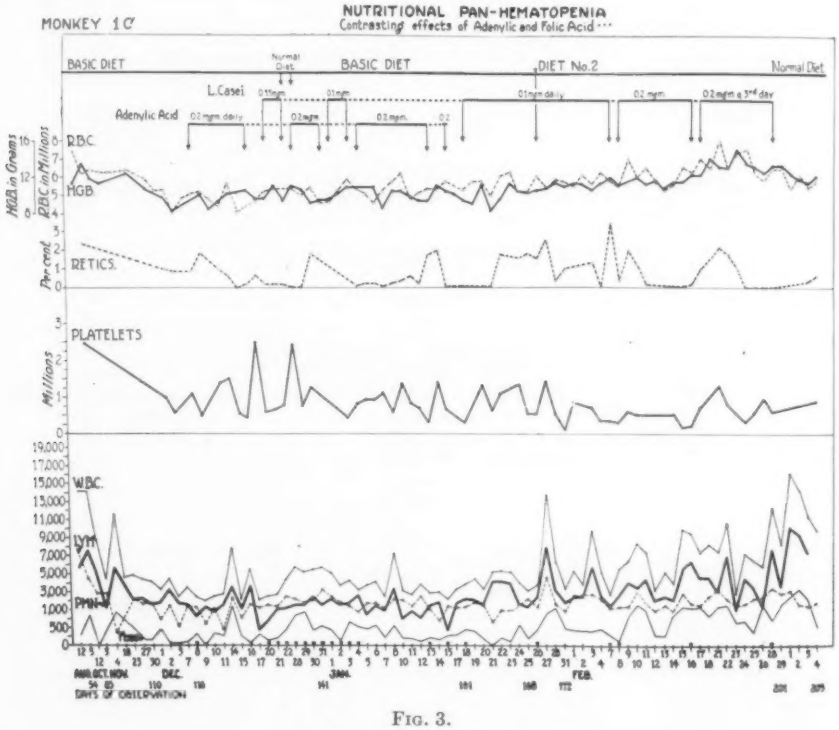

FIG. 3.

The contrast between the cytologic and clinical improvement, which followed the reavailability of folic acid in a monkey on a supplemented basic diet, II, and the fatal reaction to influenza virus infection during a leukopenic relapse is indicated in Figure 4 (M 53). Following the withholding of the folic acid concentrate after its specific and generally favorable effect on the blood cell equilibrium, appetite, weight and general appearance and activities of the animal, the cellular disequilibrium again developed, the physical condition deteriorated gradually, and when influenza virus was introduced intranasally an acute episode was precipitated resulting in death 8 days later with profound leukopenia in the presence of neutralizing antibodies. Similar fatalities resulted when hemolytic streptococci were administered under the identical circumstances (Fig. 5), even though agglutinins, opsonins and antistreptolysins developed promptly.

The monkeys, as their leukopenia developed on an inadequate diet, not only became abnormally susceptible to superimposed virus or/and pyogenic infections, they likewise became susceptible to their own endogenous, potential pathogens, more particularly those in the intestinal tract. In Table 4 are recorded the fatalities due to infection in the 35 monkeys subjected to dietary deficiency studies. It will be noted that 40% were due to spontaneous infections, the great majority succumbing to bacillary dysentery.

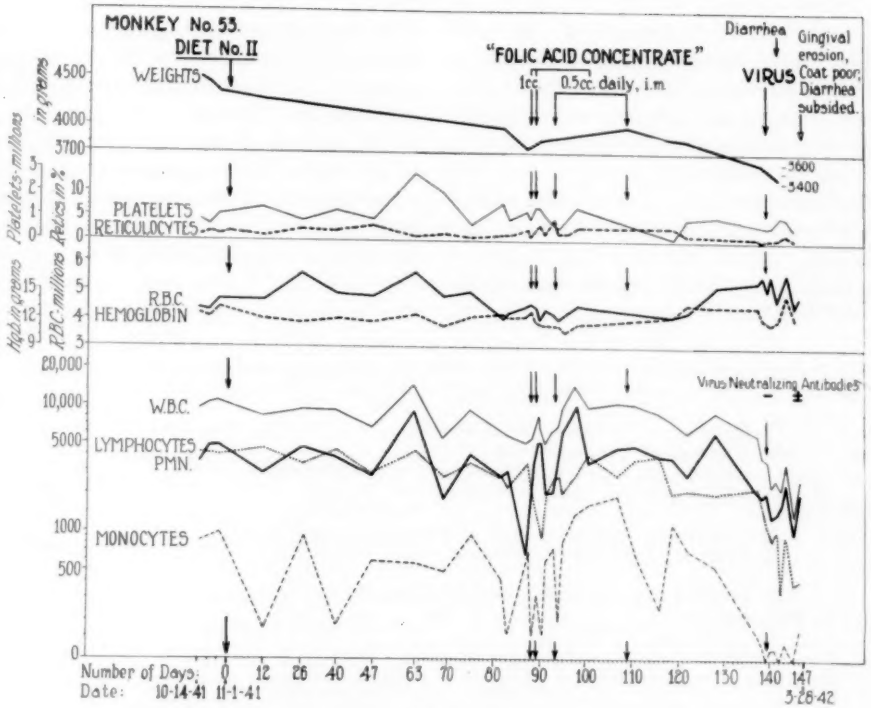
MONKEY No. 53.
DIET No. II
"FOLIC ACID CONCENTRATE"
1cc 0.5cc. daily, i.m.
Diarrhea
VIRUS
Gingival erosion, Coat poor; Diarrhea subsided.
WEIGHTS
PLATELETS
RETICULOCYTES
R.B.C.
HEMOGLOBIN
W.B.C.
LYMPHOCYTES
PMN.
MONOCYTES
Virus Neutralizing Antibodies
Number of Days:
Date: 10-14-41 11-1-41 5-28-42

FIG. 4.

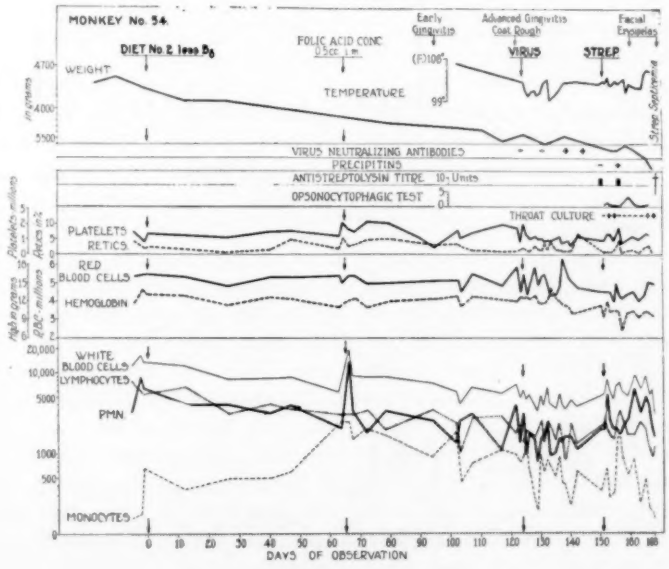
MONKEY No. 54.
DIET No. 2 less B6
FOLIC ACID CONC. 0.5cc i.m.
Early Gingivitis
Advanced Gingivitis Coat Rough
Facial Eruptions
VIRUS
STREP
WEIGHT
TEMPERATURE
VIRUS NEUTRALIZING ANTIBODIES
PRECIPITINS
ANTISTREPTOLYSIN TITRE
OPSONOCYTOPHAGIC TEST
THROAT CULTURE
PLATELETS
RETICS.
RED BLOOD CELLS
HEMOGLOBIN
WHITE BLOOD CELLS
LYMPHOCYTES
PMN.
MONOCYTES
DAYS OF OBSERVATION

FIG. 5.

Thirty % did not survive influenza virus or streptococcus exposure, which represents 71 and 83% respectively of those which received intranasal inoculations during a nutritionally induced leukopenic state. Only 1 monkey succumbed during folic acid administration (Fig. 2, M 136) in an irreversible crisis.

Another phenomenon of definite significance was observed among a few of our monkeys, which received superimposed intranasal inocula of streptococci preceded or followed by influenza virus. In Monkey 12 (Fig. 6), some 80 days following the spontaneous recovery from an initial streptococcus-virus sequence, reinfection was accomplished with the same strains of pathogens and in the same order and dosage. After a latent period of 15 to 20 days all of the signs and symptoms of an acute diffuse glomerular nephritis developed, including anorexia, albuminuria (100 mg. %), hematuria, edema and hypertension (systolic 185 mm.; diastolic 140 mm. Hg). The high opsonic index had been sharply neutralized by the re-

TABLE 4.—MORTALITY FROM SPONTANEOUS AND INDUCED INFECTIONS (35 MONKEYS) (MACACA MULATTÆ ON MODIFIED VITAMIN B DEFICIENT DIETS)

	Fatalities
I. Spontaneous *Sh. dysenteriæ* (Flexner)	11
II. Spontaneous *Kl. pneumoniæ*	1
III. Spontaneous hemolytic streptococcus bronchopneumonia (fatal)	1
IV. Spontaneous staphylococcus septicemia	1 (40%)
V. Experimentally induced hemolytic streptococcus (Group C)	5
VI. Experimentally induced influenza virus A (Strain PR 8)	5 (30%)
VII. Controls receiving liver extract or folic acid	1
VIII. Survivors	10 (29%)

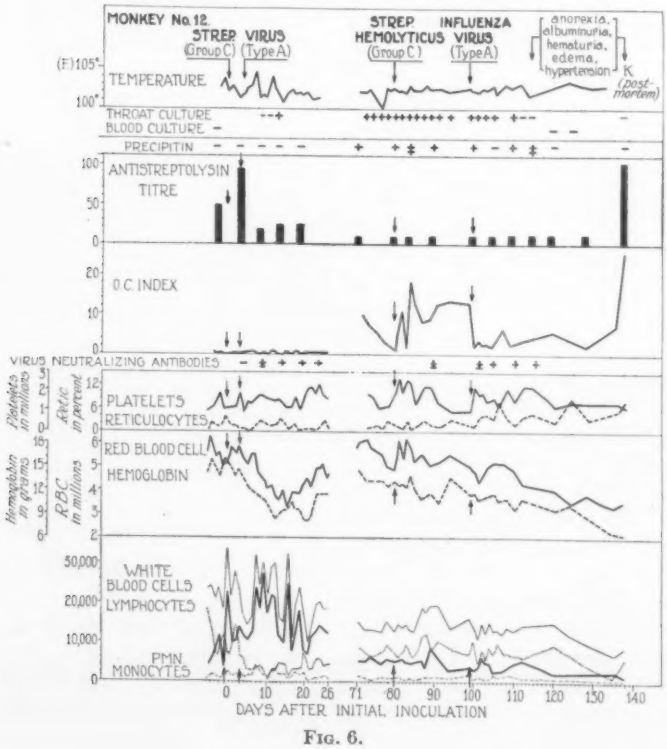

FIG. 6.

inoculation of the virus and there was a progressive anemia and leukopenia at this time, with only a slight reticulocyte response. Throat cultures were positive, but blood cultures were negative for hemolytic streptococci, and the hyperergic character of this renal episode was supported by the pathologic findings in the kidneys at postmortem. The same type of episode occurred in 2 monkeys on a deficient vitamin B diet in which spontaneous endogenous infection had occurred.

The studies of the relative susceptibility of *Macacus mulatta* monkeys, under a variety of nutritional and environmental conditions, to influenza virus and/or hemolytic streptococcus exposure *via* the nasopharynx, have once again emphasized the important rôle of the white blood cells in the body defense against infectious diseases. Furthermore, folic acid concentrate (*L. casei* factor) is at least one of the essential foods for the maintenance of the normal cellular integrity of bone marrow in the monkey, and seems to support panhematopoiesis more specifically and effectively than any other molecule thus far isolated and tested.

Clinical Studies with L. casei Factor, Synthetic. Beginning with Wills'[59] discovery that marmite, an autolyzed yeast product, was as effective as liver extract in the treatment of tropical macrocytic anemia, followed by the demonstration of its efficacy in the macrocytic anemias of idiopathic steatorrhea,[55] tropical sprue,[3] and, when given in sufficient dosage, in pernicious anemia,[62] the search for chemically active hematopoietic fractions from both liver and yeast has never ceased. The preliminary attempts to apply the experimental results with folic acid concentrates derived from each of these sources to the treatment of human anemias and leukopenias were equivocal and unconvincing, despite their striking influence on the metabolism of bone marrow in experimental animals. Moore and his associates[31] in 1943 attempted the assay of both crude and more highly purified "folic acid" concentrates in typical pernicious anemia patients, always obtaining a submaximal response, which was attributed to "some extrinsic factor activity." Castle *et al.*[4] reported negative results in relapsing pernicious anemia patients, when vitamin-free casein plus all crystalline members of the vitamin B complex, including "folic acid and folic acid concentrate" in amounts of 2.3 to 3.6 mg., were added to normal gastric juice and fed daily. Sharp *et al.*[45] obtained "an appreciable increase in the hematocrit but only slight changes in other erythropoietic phenomena" in 10 otherwise refractory anemia patients given 0.6 to 1.5 mg. of vitamin B_c in yeast concentrate. Watson[57] was unable to confirm these findings in his patients with refractory anemia, when 5 mg. of *L. casei* factor were administered daily by mouth, but suggestive responses were observed in 7 patients, who had developed leukopenia following roentgen ray therapy.

In confirmation of Watson's observations, Major Perk Lee Davis[11a] between October 1942 and July 1946, as part of a coöperative research program with Dr. Herman Hoster and our laboratory group in Columbus, observed 191 cases at the Walter Reed Hospital, Washington, D. C., diagnosed as lymphoblastomata. Included were reticulum cell sarcoma, lymphosarcoma, giant follicular lymphoblastoma, and Hodgkin's syndrome. "Complete blood and bone marrow studies revealed marked depression of both myelopoietic and erythropoietic activity. With the introduction of radiation therapy, both anemia and neutropenia became progressively more severe, necessitating daily to weekly transfusions of whole blood and/or washed red cells." Daft and Sebrell, of the National Institute of Health, were instrumental in securing, through the courtesy of Dr. Thomas Jukes of the Lederle Laboratories, sufficient quantities of folic acid to permit treatment of selected patients in this Army group with large oral doses of 75 to 150 mg. t.i.d. "Seventeen cases, given these large doses of folic

acid without any other therapy, showed no demonstrable beneficial influence on the course of the disease. Sixty-nine cases were then treated with radiation therapy, transfusions and folic acid. When folic acid was added to the radiation therapy, it was noted that the patients withstood radiation better, with less nausea, vomiting and general depressing physical effects. The necessity for red cell or whole blood transfusions decreased in frequency to an average of one in 18 days. The average time of hospitalization was reduced from 9.8 to 5.3 months. The bone marrow on discharge showed an increase in the myeloid and erythroid elements." Davis concludes: "Folic acid is recommended as a useful adjuvant in maintaining the hematologic well-being of patients receiving massive and prolonged radiation therapy."

Zuelzer and Ogden[64] have just reported 12 infants under 1 year of age with severe macrocytic anemia and megaloblastic bone marrows of unknown, non-dietary deficient etiology, 9 of whom responded promptly and completely to *L. casei* factor concentrate, or synthetic. The dosage was 5 to 20 mg. per day for 8 to 21 days. Bone marrow, reticulocyte and hemoglobinogenesis curves were "virtually identical with those obtained in pernicious anemia following adequate treatment with purified liver extract." Completely negative results are reported in the anemia of prematurity (18 cases), hypochromic microcytic anemia (8 cases), chronic hypoplastic anemia (2 cases), and in 1 patient each with diagnoses of Mediterranean anemia, sickle cell anemia, aplastic anemia, subacute myelogenous leukemia, and acute lymphatic leukemia, respectively.

Encouraged by the consistent and spectacular response of specifically leukopenic or pancytopenic monkeys to "folic acid concentrates" in our laboratory,[39,61] an attempt was made, in coöperation with Dr. Spies beginning in the summer of 1943, to select certain patients at the Hillman Hospital, Birmingham, with nutritional deficiency reflected by leukopenia and mouth ulcers, and to appraise the value of *L. casei* factor, first in the form of the purified crystalline concentrate from liver, later as the synthetic molecule. Controlled peripheral blood and bone marrow studies were made, using the supravital technique, and both hematologic and clinical improvement, commensurate with the dosages then available, were reported.[2]

Table 5.—Summary of 28 Cases Treated With L. Casei Factor, Synthetic

I. *Satisfactory:*		
1. Addisonian pernicious anemia		10
Virgin case	1	
In relapse	3	
Maintenance	3	
Hypersensitive to liver	3	
II. *Unsatisfactory:*		
2. Hypoplastic marrow		6
Primary	4	
Secondary	2	
3. Monocytic leukemia		5
Chronic	4	
Acute	1	
4. Acute influenza virus infection, with profound leukopenia		3
5. Macrocytic anemia, secondary to hepatic cirrhosis		1
6. Hemolytic anemia, acquired		1
7. Myelogenous leukemia, chronic		1
8. Lymphatic leukemia, chronic		1

In November 1945, and each month since, Spies and associates[48–51] have presented a rapidly increasing series of patients with macrocytic anemia, who have responded dramatically and completely to parenterally and orally administered *L. casei* factor, synthetic. These cases have included nutritional deficiency *per se*, Addisonian pernicious anemia, and the macrocytic anemias of pregnancy,

pellagra and sprue, both tropical and nontropical.

Coincidentally and independently, Darby and Jones[11] obtained satisfactory hematologic and clinical remissions in 2 patients with sprue, using 15 mg. of the same synthetic material, intramuscularly, daily. Moore and associates[31] and our own group[21] immediately confirmed, and have since paralleled and elaborated upon the reported clinical findings of the Birmingham and Nashville groups.

Table 5 summarizes the 28 patients thus far studied in our Clinic together with their respective diagnoses. Figures 7, 8 and 9 present the essential dosage requirements and bone marrow and peripheral cellular responses elicited by synthetic folic acid (*L. casei* factor) in 3 individuals with classical Addisonian pernicious anemia. The changes in the sternal bone marrow were particularly closely followed in the patient whose laboratory studies are correlated in Figure 7. Folic acid (2 mg. synthetic) was given intravenously for 20 consecutive days. A prompt and rapid maturation of the megaloblasts was observed with the restoration of an approximately normal cellular maturity pattern by the 10th day, the maximum reticulocyte response (26.8%) being recorded on the 15th day of therapy, after a total cumulated dosage of 30 mg. folic acid. Increasing the dose to 10 mg. daily beginning with the 16th day failed to induce a secondary reticulocyte rise, confirming the adequacy of the original dosage. On the 40th day of therapy the total red cells reached 4,000,000, hemoglobin 13 gm. from a low of 1,000,000 erythrocytes, hemoglobin 5 gm. Clinically on the 2nd day of therapy this patient volunteered that the paresthesia of the extremities was definitely diminished with a very evident improvement in the subjective sense of well-being; the vibratory sense and the Romberg were objectively improved by the 21st day of therapy. These findings are identical with those observed and reported previously in patients with pernicious anemia receiving optimal parenteral liver extract therapy.[23] This patient has been maintained in hematopoietic equilibrium and abundant health on 20 mg. of synthetic folic acid at fort-

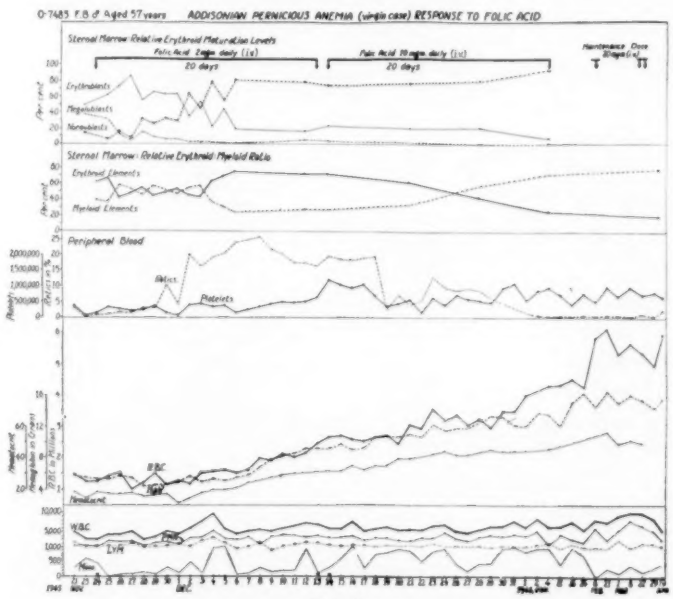

FIG. 7.

nightly intervals since the remission was established.

While 2 mg. orally of folic acid daily were insufficient to appreciably increase the peripheral red cell level after 10 days (Fig. 8) in a white female patient with pernicious anemia, in relapse, the megaloblasts in the sternal bone marrow were reduced from 45 to 24% during this period and almost immediately following the increase to 10 mg. daily by intramuscular injection, a reticulocytosis developed (22%), and 25 mg. daily, later, and 100 units of liver extract failed to induce

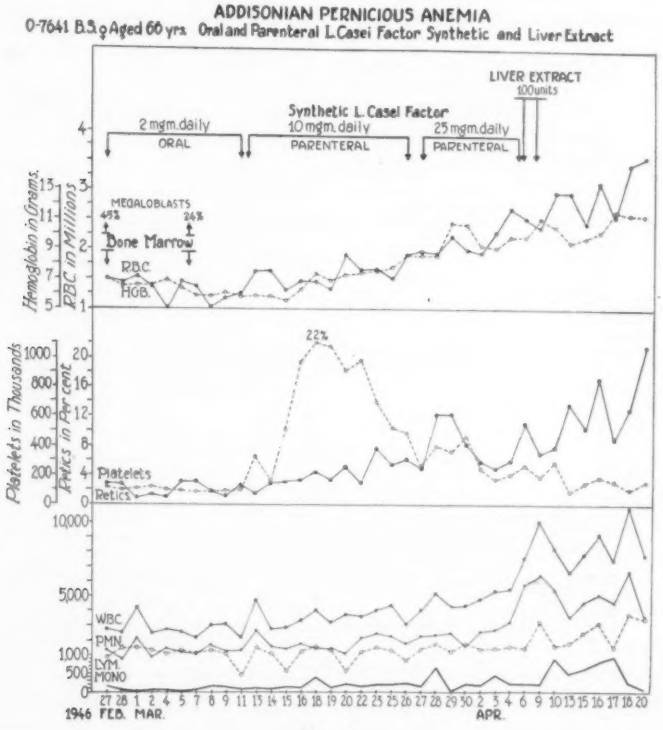

FIG. 8.

any significant secondary reticulocyte peak. The chief complaint on admission in this patient was severe angina pectoris, several attacks daily requiring nitroglycerine for relief. There have been no subjective or objective cardiac manifestations since the hemoglobin improved.

Again in Figure 9 it will be apparent that 5 mg. of folic acid given orally daily to this patient with pernicious anemia in relapse were suboptimal as revealed when the dosage was raised to 50 mg. with a secondary reticulocyte response.

In 1 patient (Fig. 10) a white female, aged 56, with advanced hepatic cirrhosis and ascites and with a long alcoholic history, we have observed over a period of years a chronic macrocytic anemia with moderate leukopenia resistant to therapy. The hippuric acid excretion is currently 1.49 gm., prothrombin 28%, cephalin flocculation test +++. The erythrocyte MCV is 105 cμ, MCH 27, and the sternal marrow is hyperplastic with an erythroid predominance showing a moderate "left shift" pattern with 5% megaloblasts, 17% early erythroblasts, 22% late erythroblasts and 58% normoblasts. Folic acid, oral and parenteral, has failed thus far to mature these elements or correct the macrocytosis as have 100 units of liver extract concentrate par-

enterally. The rôle of the liver in the utilization of folic acid may be suggested should similar macrocytic anemias with megaloblastic arrest secondary to far advanced hepatic cirrhosis continue to be refractory to this stimulus.

The combined studies have conclusively established at least 2 important and extremely significant points: (1) that *L. casei* factor contains a potent anti-anemia component completely effective in a variety of human macrocytic anemias; (2) that all of the human macrocytic anemias thus far studied, including Addisonian pernicious anemia, reflect a common mechanism of megaloblastic maturation arrest, not *primary* or *specific* to any one syndrome, but in every instance *secondary* to some interference in the availability of an essential nutritional complex.

The effective dosage of *L. casei* factor synthetic in a fully developed, advanced macrocytic anemia varies from patient to patient, from as little as 1 to 25 mg. per day. It has been proven to be therapeutically effective when given by any route; oral, parenteral, per rectal enema (100 mg. per day).[32] In several hypoplastic anemia patients, in whom we desired to give a maximum single intravenous daily dose, we found that 125 to 150 mg. represented the top tolerance level without unpleasant subjective histamine-like, vasomotor symptoms. Spies[50] reports no untoward reactions to 400 mg. daily by mouth. Maintenance dosages in patients with pernicious anemia in full remission have thus far varied in our clinic between 20 and 40 mg. at 1 to 3 week intervals. Minor neurologic signs and symptoms in patients in relapse have responded as promptly and completely following *L. casei* factor supplements as with potent liver extract, and no progressive cord lesions have as yet been noted. In those of our patients who have developed hypersensitivity to all liver products, *L. casei* factor synthetic has been substituted in dosages

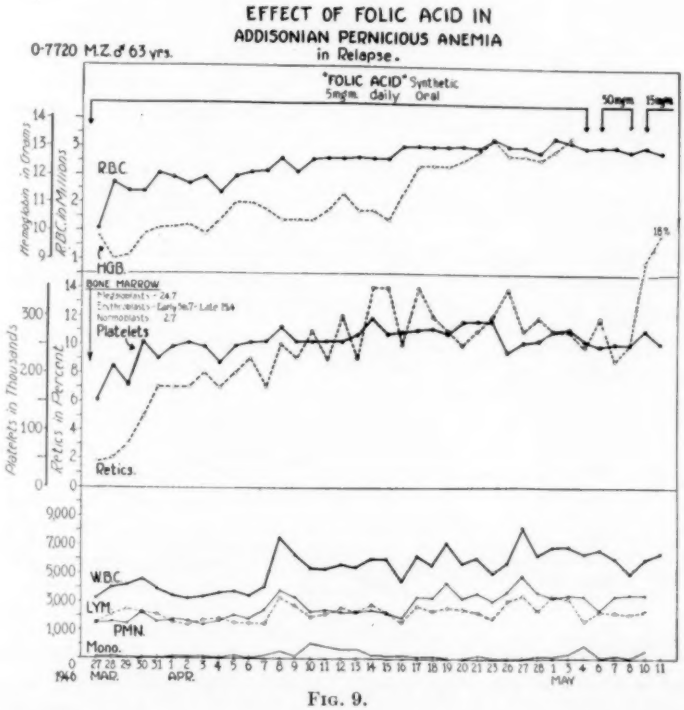

FIG. 9.

sufficient to maintain an optimum hematopoietic equilibrium with, in no instance, any intolerance or allergic manifestations to date. Idiopathic marrow hypoplasia, myelophthisic anemia, the leukopenia of virus infections, and the iron deficiency states do not, and would not be anticipated to respond (Table 5). Unfortunately, also the maturational stimulus for normal leukopoiesis has failed to affect the immature white cells in the acute

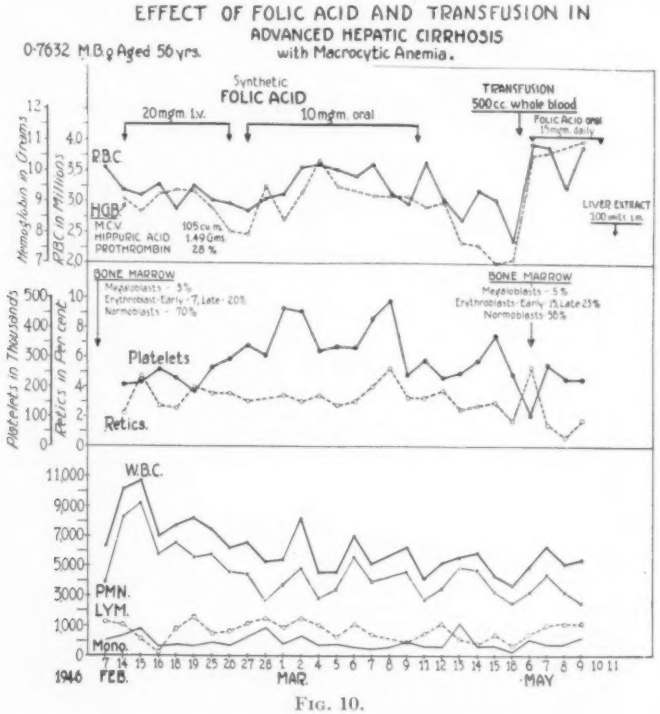

FIG. 10.

effective by mouth in relatively small volume in patients with classical pernicious anemia having diminished or absent intrinsic factor; (2) it is effective when given parenterally; and (3) its activity is not appreciably augmented by incubation with normal human gastric juice. Is it the active anti-anemia principle in liver and liver extract? It would seem unlikely, since: (1) the minimum effective dose is 1 mg., usually 4 to 10 mg. daily, while

and subacute leukemias, in a limited experience, but a principle has been established for further elaboration and exploration in this area.

There is naturally much speculation as to the precise rôle played by *L. casei* factor in erythropoiesis and pan-marrow hematopoiesis. With the exact chemical formula still unrevealed,* analogies still may be drawn between this factor and the other known, essential marrow stimulating agents. Does it act as "extrinsic factor?" Probably not, since: (1) it is

potent purified fractions of liver "containing only 0.035 to 0.7 mg. *of material* per injection"[54] have produced adequate hematologic effects; (2) *L. casei* factor assay has revealed 0.02 to 0.5 mg. only per U.S.P. unit of liver extract;[5] (3) the minimal effective oral dose approaches the minimal effective parenteral dose; (4) no evidence of antigenic sensitization has yet developed following folic acid. It must be remembered, however, that, with the recognition of *L. casei* factor conjugates reducible by enzymatic action with the

* "Pteroylglutamic Acid," Science, **103**, 6, 1946 (May 31).

release of "free" factor, the mere presence in food or gastro-intestinal tract of "folic acid" need not mean it is in a utilizable form. Moore[31] suggests that the intrinsic factor of Castle may be concerned with the release in man of the *L. casei* factor from its combined form. Spies[50] offers the hypothesis that the anti-anemic factor present in liver and yeast is either a much larger molecule than *L. casei* factor, or represents a more potent or effective combination of chemical substances per unit volume. The precise explanation awaits further evidence.

Still more recently Spies[51] has reported the erythropoietic effectiveness of 2, 4-dihydroxy-5-methyl-pyrimidine synthetic (thymine), in dosages of 4.5 to 20 gm., oral administration, daily with reticulocyte response and red blood cell recovery in human macrocytic anemia. Thymine is a normal constituent of body cells and has been shown to support the growth of *L. casei*. While the quantitative requirements are much greater for equivalent bone marrow effects, it would seem probable that this, too, represents one more link in the same chain of evidence, which is being currently accumulated.

Conclusion. In analyzing the increasing volume and variety of both clinical and experimental studies with "folic acid," as derived from spinach, liver, yeast, other natural sources, and now, the synthetic *L. casei* factor, it becomes increasingly apparent that we are dealing with one of the more fundamental molecules essential to the normal metabolism of all cell types in marrow, and in young, actively growing cells and tissues generally, perhaps. In the absence of "sufficient" *L. casei* factor due to extrinsic dietary deprivation, or with intrinsic interference in absorption or utilization, a more or less marked, specific or general, cellular inadequacy develops in mammalian bone marrow with the appearance of signs and symptoms associated with, and dependent upon, the extent and specificity of the individual's needs. Many other factors, including infections, condition and modify the relative clinical stability or instability of each human organism. The alert physician must, therefore, recognize these variables, and exert the maximum of ingenuity in his search for the "balance of power" among the known or knowable factors in the equation, and when this is done, more often than not, the problem can, and will, be solved.

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PRIMARY SYSTEMIC AMYLOIDOSIS: JAUNDICE AS A RARE ACCOMPANIMENT

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Primary systemic amyloidosis is a rare pathologic entity and, although first described by Wilks in the Guy's Hospital Report for 1856 as mentioned by Dillon and Evans,[4] was only noted 24 more times in the literature up until 1939 according to Koletsky and Stecher.[8] Recently Lindsay and Knorp[10] in reviewing the literature noted 16 more cases, including 1 of their own. Eight of these had appeared before 1939. Since their paper Brown and Seltzer,[3] and Golden[6] have added 2 others.

At the Montefiore Hospital, New York City, there have been 3 cases in the last 3 years. Perla and Gross[14] in 1935 originally reported 3 others from this institution, but in retrospect their second case was probably secondary to chronic rheumatoid arthritis. Of the recent 3 cases, the first was a case of primary amyloidosis of the heart, the second of the liver, spleen, kidneys, gastro-intestinal tract and blood-vessels, and the third of the cardiac and striated muscle and of the vessels and lungs. Only the second will be discussed here because of the very rare finding of jaundice of many months duration in man with hepatitic amyloidosis and a large gastric ulcer.

Most authors, including Moschcowitz,[12] have stated that amyloidosis of the liver is either rarely or never accompanied by jaundice. Pearlman[13] and Rosenblatt[17] in a large series of cases make no mention of its presence. In a study of hepatic function in this disorder, Tiber, Pearlman and Cohen[20] noted no icterus in 30 cases personally observed and only 1 case of transient jaundice in the records of 100 patients with severe liver amyloidosis. Bannick, Berkman and Beaver,[1] however reported a case of a 44 year old man who 1 week before death developed obstructive jaundice and ascites and at autopsy had only generalized amyloidosis of the liver, spleen and other organs. There was extensive atrophy of the hepatic cords between which there was an accumulation of amyloid for which there was no apparent cause. Their third case had a bilirubin of 1.7 mg. with liver amyloidosis and a stomach carcinoma. Theirs were apparently the first such cases reported in the literature, but there was no discussion of the pathogenesis of the jaundice.

Recently Spain and Riley[18] reported another case. They reviewed 50 cases of liver amyloidosis and only noted 1 with jaundice. This was a 29 year old female with chronic pulmonary tuberculosis who had extensive liver amyloidosis and developed clinical jaundice 1 week before death. Unfortunately no bilirubin determination was done, although before that time it had been normal, as was the cephalin flocculation test. The alkaline phosphatase, though, had been elevated to 20 Bodansky units. It was their interpretation, after reviewing the slides of 35 other amyloid livers without jaundice and with less extensive amyloid replacement, that the icterus was due to both an actual numerical insufficiency of liver cells and a blockage of bile canaliculi.

The other unusual feature in our case was a large gastric ulcer. Gastro-intestinal involvement by amyloid is not uncommon and symptoms such as diarrhea and melena are often found in this condition. Golden[6] noted that Lubarsch in 1929 described a case of amyloidosis of the

stomach with pyloric ulcerations and hematemesis. His case had no free acid and the amyloid involved the muscularis and vessels. Koletsky and Stecher[8] quote Steinhaus in 1902 as having a case of amyloidosis with gastro-intestinal bleeding and involvement of the stomach. One of Perla and Gross'[14] cases had severe duodenal ulcerations. Recently Golden[6] reported a case of a 66 year old colored female who had had ulcer symptoms for many years. Examination of the stomach after gastrectomy revealed amyloidosis of the entire wall and 2 superficial ulcers near the pylorus supplied by amyloid infiltrated vessels. Lindsay and Knorp's[10] case had typical ulcer symptoms and at autopsy had a lesser curvature ulcer which they thought was on an amyloid basis.

Case Report. (M. S., No. 37542) This was the first Montefiore Hospital admission of a 44 year old male welder who had begun to complain of intermittent constipation and right upper quadrant pain in May 1943. Six months later he was admitted to another hospital because of a 15 pound weight loss and icterus. Physical examination at that time revealed only jaundice, but a gastro-intestinal *X*-ray series demonstrated partial obstruction at the hepatic flexure suggestive of neoplasm. At laparotomy only adhesions about this portion of the colon were found. The gall bladder emptied easily and contained no stones, but the liver was enlarged and peculiarly yellowish. A biopsy taken was subsequently reported as showing cloudy swelling, fatty infiltration, and dilated sinusoids with precipitated bile. Jaundice increased postoperatively and after a course complicated by a sulfonamide-responsive pneumonia the patient was discharged on a low fat diet and given injections of liver extract for 10 weeks. Seven months later, in May 1944, he was admitted to Montefiore Hospital, complaining of progressive weakness, weight loss, pruritus and abdominal distention. He also had begun to note right upper quadrant and epigastric pains following meals and relieved by Seidlitz powders. Edema and ascites had appeared 1 week previously.

Physical examination showed him to be poorly nourished and markedly jaundiced. His heart and lungs were normal. Blood pressure was 100/60. Liver and spleen were both markedly enlarged and ascites and edema were present.

Laboratory Data. The hemoglobin was 13 gm., with 4.5 million red cells, hematocrit 35.5, white count 13,600 (80% neutrophils, 12% lymphocytes, 2% basophils, 1% monocytes). Urine always showed from a trace to 2+ protein, was positive for bile and a minimal amount of urobilinogen, concentrated to 1.024 in casual specimens, and had occasional red blood cells, granular and hyaline casts. The stool gave a 4+ benzidine test but was light brown in color. Gastric analysis showed blood, free acid up to 11 units and total up to 22. Blood urea nitrogen was 12.8 mg. per 100 cc., sugar 84 mg., total protein 6.6 gm. with an albumin of 3.9 and a globulin of 2.7. The euglobulin was 0.7, pseudoglobulin I 1.2, pseudoglobulin II 0.8. Blood calcium was 10.7 mg., phosphorus 3.9 mg., bilirubin 16.3 mg., the van den Bergh reaction was direct, icterus index 71, total cholesterol 512 mg. with an ester fraction of only 112 mg., alkaline phosphatase 36.6 Bodansky units, cephalin flocculation negative, prothrombin time 23 seconds on whole blood and 63 on a dilution of 1 to 8. The Exton-Rose 2-dose glucose tolerance test showed a fasting blood sugar of 83 mg., 95 in 30 minutes, and 102 in 1 hour. Urea clearance was 73%. Hippuric acid excretion was 2.39 gm. in 4 hours. Urine was negative for melanin. The 24 hour 17-ketosteroid excretion was markedly depressed to 0.9 mg. The test for syphilis was negative. The abdominal fluid had a total protein of 2.6 gm. per 100 cc. Electrocardiogram showed left axis deviation. Roentgen ray of the chest demonstrated basal atelectasis, elevation of the diaphragm, and dorsal kyphoscoliosis. The gastro-intestinal Roentgen series showed a large gastric ulcer on the lesser curvature which was confirmed by gastroscopy. Thorotrast was not visualized in either liver or spleen by Roentgen ray after intravenous injection. Barium enema and long bone Roentgen rays were normal. No Congo red test was done.

Course. While no definite diagnosis was achieved, it was suspected that the patient

had a gastric malignancy with liver metastases. Supportive therapy did little to affect the progressive downhill course, and the patient died 1½ months after admission.

AUTOPSY. The anatomic diagnoses were: primary amyloidosis of the liver, spleen, kidneys, adrenals, heart, pancreas, lymph nodes, testes, small arteries and veins; thrombosis of the portal, splenic and superior mesenteric veins; thrombi in small branches of the pulmonary arteries and veins; jaundice; ascites; edema of the lower extremities; hypertrophy and dilatation of the heart; slight fatty degeneration of the heart; congestion and edema of the lungs; bronchopneumonia; large chronic ulcer of lesser curvature of stomach.

All of the above-mentioned organs showed the typical gross amyloid stain with iodine. The liver weighed 5900 gm. and was large, firm, friable and pale yellowish brown in color. On section the cut surface had a dull glistening appearance. The liver lobules were distorted and there were small areas of hemorrhage. The gall bladder contained no stones, had patent ducts, and bile was easily expressed into the duodenum. The spleen weighed 1200 gm. and was also yellowish brown with hemorrhagic areas. The stomach was markedly dilated with absent rugæ. There was a large gastric ulcer, 5 cm. in diameter, on the lesser curvature.

Microscopic examination of the liver revealed extreme amyloidosis with obliteration of most of the sinusoids and replacement of most of the parenchyma. Only scattered remnants of liver cords or small bile ducts remained in places. The small arteries and arterioles showed marked amyloid thickening with narrowing of the lumen. There was also an organizing thrombus in a large branch of the portal vein. In a very few foci there were bile thrombi present. Congo red stain on liver sections was positive. Sudan III stain showed fatty degeneration of remaining liver cords.

The stomach section was taken at the edge of a deep ulcer which had extended through all the layers of the wall. It was of usual chronic type with well-formed granulation tissue, liver adhesions, foci of hemorrhage, and infiltration by plasma cells, lymphocytes and eosinophils. Many of the arteries in the ulcer base showed subintimal thickening and thrombi. There was present what was probably amyloid deposition about some of the capillaries in the mucosa and in the walls of small arteries in the submucosa. The muscle bundles in the muscular layers were separated by fibrous tissue or amyloid.

The rest of the autopsy revealed generalized amyloidosis as evidenced by iodine and Congo red stains as well as the microscopic picture.

On reviewing the original biopsy taken at the other institution it was found that it too demonstrated extensive amyloidosis and fatty degeneration.

Discussion. The overall picture of cachexia, hepatosplenomegaly, obstructive jaundice and melena naturally suggested a diagnosis of gastro-intestinal malignancy with liver metastases and when this was not confirmed at autopsy it came as a complete surprise.

It is generally stated in the literature[8] that primary amyloidosis affects mainly mesodermal tissues, the gastro-intestinal tract, smooth, skeletal and cardiac muscles; that it stains atypically, and that it is unassociated with any known etiologic agent. However, as in our case, many[8,13,15] have noted amyloidosis of primary or idiopathic origin distributed in the parenchymal organs as is more usual in the secondary type disease.

The rarity of jaundice in amyloidosis has been well emphasized. Hamilton,[7] Drinker[5] and others[2] have never seen amyloidosis result from welding, although transient jaundice occurred on at least 1 occasion. Among 2260 autopsy protocols at Montefiore, there were 102 cases of amyloid disease, none of which had jaundice. Lichtman[9] goes so far as to say that when jaundice does occur it is due to concomitant cirrhosis or gumma.

Why jaundice occurred in our case, as well as in Bannick's and in Spain's, is not clear. The latter's contention, that it is due to bile canaliculi obstruction plus actual cellular insufficiency, is supported by the paucity of liver cells in our case, the pressure atrophy, bile thrombi and the

enormous weight of the liver (5900 gm.) in comparison with the weights of the 102 livers without jaundice (none over 3300 gm.). Unfortunately, however, this concept does not explain the absence of jaundice in similar cases, since other amyloid livers noted at Montefiore had as marked a numerical insufficiency of cells microscopically.[16]

The quantitative source of bilirubin (hemoglobin turnover) as well as the activity of the manufacturing cells would seem to be of importance in the pathogenesis of jaundice. Many of these patients are profoundly anemic so that the low hemoglobin as a limiting source of bilirubin, when coupled with a deficiency of reticulo-endothelial cells which ordinarily form bilirubin, might explain the absence of jaundice. Our patient, in contrast to most cases of amyloidosis, had a practically normal hemoglobin. On the other hand, his reticulo-endothelial system was seriously depleted as shown by microscopic replacement of lymph nodes and spleen with amyloid and the absence of Küpffer cells in the liver. Thorotrast visualization according to the method of Yater,[22] *et al.* failed. The interpretation of this might well be that there were not enough reticulo-endothelial cells present to take up thorotrast in sufficient concentration to cause Roentgen ray visualization. Apparently, then, the presence or absence of jaundice in most cases of amyloidosis may depend upon a balance between a sufficient source of bilirubin, active reticulo-endothelial cells to form it, and bile canaliculi obstruction. Most often there is no jaundice because of anemia, destroyed reticulo-endothelial cells and insufficient amyloid obstruction.

It is interesting to note the dissociation in liver function studies in our case. The elevations of bilirubin, alkaline phosphatase and total cholesterol all pointed to obstructive jaundice, while the diminished hippuric acid synthesis, low cholesterol ester and prolonged prothrombin time suggested parenchymatous disease. Despite the latter, the cephalin flocculation test, one of the most sensitive indicators of hepatocellular dysfunction, was negative. Perhaps this speaks for an insufficiency of gamma globulin as suggested by Moore *et al.*[11] to explain negative tests.

Gastro-intestinal infiltration by amyloid is generally extensive in so-called primary amyloidosis, both in the muscularis and nutrient blood-vessels. When the latter are severely affected, mucosal ulceration might conceivably appear. In our case it was not too obvious and the ulcer could just as well have been of the usual idiopathic type.

It is quite obvious from a review of the literature that the clinical recognition of primary amyloidosis is even rarer than the pathologic entity. Many have emphasized the possibility of amyloidosis in obscure hepatosplenomegalies, cases with macroglossia,[21] and anemia. Now, perhaps, unexplained jaundice should also be viewed with suspicion and a Congo red test[19] performed. Unfortunately, especially in cases of primary origin, this may be negative and the diagnosis can then only be made on biopsy.

Summary. 1. A case of primary amyloidosis with jaundice and a gastric ulcer is presented.

2. The rarity of jaundice in amyloid disease of the liver is emphasized by the few instances (4 cases) in which that association has been reported.

3. The possible pathogenesis of jaundice and gastric ulcer in amyloidosis is discussed.

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POSITIVE REACTIONS TO THE KAHN TEST FOR SYPHILIS—THEIR INCIDENCE AND MEANING IN HEALTHY AMERICAN MEN

A SURVEY OF 82,070 U. S. MARITIME SERVICE ENROLLEES

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WHEN a healthy American male exhibits a positive serologic test for syphilis, what is the likelihood of his having the disease? The present report presents one answer to this question in terms of experiences with the Kahn test at a large semi-military training station. Summarized are the serologic findings and their clinical interpretations in a well-studied series of 82,070 male Maritime Service enrollees. The quota of false positives and sero-positive syphilis, congenital as well as acquired, may be assumed to be fairly typical of conditions existing in the comparable adult male population of this country, within the limits of age, race and geographic distribution.

World War II has been responsible for the performance of millions of serologic tests for syphilis on Selective Service registrants and recruits of the Army, Navy and other fighting forces. A number of statistical tabulations of the sero-positive reactors in various states and fractions of states[2,4,6,7,10,12,14,15,18,19] are already on record. The largest series as yet reported has been that of Vonderlehr and Usilton,[18] who in 1942 collected and tabulated nearly 2,000,000 serologic reports from Selective Service sources. The prevalence of syphilis for the whole country over the age period, 21 through 35, was calculated as 45.3 per 1000. This figure was based exclusively upon positive and doubtful blood tests. The true rate was then estimated as being closer to 47.7 per 1000, to allow for the fact that not all syphilitic cases give positive blood responses. The prevalence of false positives was not evaluated. Lowest rates for syphilis were found for these areas in which most of our own subjects were recruited, namely, New England, the North Central, and the Middle Atlantic groups of states.

That serology *per se* cannot be depended upon to pick out every case of syphilis, was well brought out in the 1941 evaluation of State Laboratories.[11] The criterion of skill, as chosen, was the ability to demonstrate positive reactions in blood from known syphilitic subjects. In success these laboratories ranged from 61.3 to 81.3% with the Kahn Standard test; from 65.6 to 83.9% with complement fixation techniques; and from 70.3 to 83.5% with the Kline Diagnostic precipitation test. The existence of a sero-negative fraction within the total population must be kept in mind when considering statistics gathered from serologic sources.

The present survey differs from most of the others. More attention has been devoted to distinguishing between true and false positive reactions and between acquired and congenital syphilis. All interpretations of sero-positivity are based upon careful clinical work-ups by a well-organized venereal disease clinic.

Modern syphilologists are trained to view every sero-positive individual with the highest suspicion. This aggressive approach embodies the accumulated tradition of American medical thought, and through the years has proved highly efficient in stimulating the detection of latent cases needing treatment. Since the outbreak of World War II, however, the awareness is growing that diverse other conditions often give rise to non-syphilitic positive responses. As a conse-

quence, it is now being counselled that sero-positive individuals who are negative clinically should not receive antisyphilitic treatment unless their blood reactions remain constant for a minimum of several months without drop in titer; and then only when all other known causes of false positive reactions have been painstakingly excluded.[17]

Blood reactions positive from causes other than syphilis fall into 2 logical divisions: those due to faulty technique in collection or testing of the specimens (usually called "technical false" positives); and those due to non-syphilitic reactive substances actually present in the serum (usually called "biological false" or "non-specific" positives).

Positives resulting from faults in collection or testing originate from mislabelling, bacterial contamination, hemolysis due to improper collection and storage, faulty controls, inaccurate readings, defective reagents, the addition of oxalate or citrate, clerical mistakes, and so forth. It has been customary to ascribe to such causes those instances in which a positive reaction of strong or weak intensity is encountered only once. Such usage merits serious reconsideration in the light of recent observations that the weak sero-positivity associated with non-syphilitic ailments may last but 1 or 2 weeks.

Positive reactions of non-syphilitic origin may be associated with any one of a wide variety of infectious diseases—among which may be mentioned yaws, leprosy, malaria, infectious mononucleosis, measles, upper respiratory infections, vaccinia, and the bacterial and "viral" pneumonias. Kolmer,[8] Davis,[3] and Beerman[1] have recently re-evaluated the immunologic literature on this subject. "Attempts to find consistent empirical physico-chemical differences between syphilitic and false positive sera have thus far failed."[3]

The first large-scale analysis of the incidence of non-syphilitic positive reactors among otherwise normal individuals was that of Eagle.[5] In 1941 Eagle reported on screening tests of 40,545 male and female students in 15 American colleges. The initial testing uncovered 190 positive specimens, but 128 could not be confirmed by a second blood examination. These 128 were classified arbitrarily as due to laboratory errors in technique, possibly without significant justification. The interval between tests was not stated.

Of the 62 students whose responses remained persistently positive or doubtful, 26 were found to be either actively syphilitic or recipients of previous antisyphilitic treatment. Remaining were 36 "doubtful" students with no clinical signs or known history of syphilitic infection, a prevalence of 1 in 1125 (0.09%). Of these, 15 came from 7 schools (5783, total enrollment) which had 11 cases of syphilis. At the other extreme, in 10 schools (11,287, total enrollment), with no proved case of syphilis, only 1 student had a positive serologic test.

To these relationships, the author applied the coefficient of correlation and derived a "corrected" prevalence for biologic false positive reactions of "less than 1 in 4000." This "corrected" estimate has received wide acceptance in text-books and reviews, but the methodology employed may be subjected to criticism. Not all 36 members of the "doubtful" group were followed for a 3 to 6 month period to see what happened to their serologic reactions. The largest college had 6 syphilis and 12 "doubtful" cases, an incidence greatly different from that of the other schools. If this outlying datum had been excluded from the calculations, only the slightest relationship between the 2 kinds of responses would have been demonstrable. Nor does a given correlation coefficient between 2 serologic variables indicate that a definite percentage of 1 belongs with the other. In fact, there is a grave question, statistically speaking, whether the coefficient of correlation technique is applicable to such data. To report the initial prevalence value as 1 in 1125 would seem more advisable than to introduce this corrective computation of doubtful validity.

Mention may be made also of the report of Stokes *et al.*[16] on the serology findings among 210,261 Red Cross donors tested at the Philadelphia Blood Donor Center in 1944. Only 489 (0.23%) gave definite positive reactions to serologic tests for syphilis, of whom 59.5% were adjudged, after exhaustive study, to be of a non-syphilitic nature. The prevalence of such non-syphilitic positive reactions, therefore, averages 1 in 708 in this series.

Procedure. The 82,070 subjects of our study were recruited in the northeastern United States, in the segment extending from New England down to and including North Carolina and as far west as the Mississippi River. The series comprised every basic-training enrollee reporting between June 1, 1943, and July 1, 1945. Approximately 60% were between 16 to 20 years of age, and another 30% were between 21 and 30; their age distribution is indicated in Tables 2 and 3. They came from every economic and social level. Nearly all were white. Preliminary serologic testings at the time of their enlistments were not done, and the majority had never previously been given a "blood test."

Since the Kahn test is utilized by the United States Navy and procedures within the Maritime Service are in general patterned after those of the Navy, the Kahn test was adopted for the serologic screening of these men for syphilis. Conclusions drawn with regard to sero-positive reactions are, therefore, based upon the results of this test.

Blood specimens were taken within 48 hours of arrival. The specimens were subjected to the Kahn Standard test, or to the Kahn Presumptive test, followed by the Standard test on all suspicious and positive reactions. These tests were done, as a rule, on the 1st or 2nd days after collection, although uncontrollable factors at times made delays necessary. Whenever the reaction was positive, doubtful, or difficult to read, the Kahn test was repeated. At the same time a duplicate specimen of blood was sent to the Venereal Disease Research Laboratory of the U. S. P. H. S., U. S. Marine Hospital, Staten Island. There the sera was checked by a serologic "battery" of 7 precipitin and complement fixation tests —the Kahn Standard, the Kolmer Complement Fixation, the Kline Diagnostic, the Kline Exclusion, the Eagle Flocculation, the Hinton Flocculation and the Mazzini Flocculation tests.

The serologic "battery" furnishes a broader picture of the serum reactivity patterns than any single test procedure. Sera with a strong content of reactive substances are positive to all tests. Positive sera from treated and non-syphilitic individuals often give weak, doubtful, or negative readings with some of the tests and negative findings with others. Mahoney[9] has pointed out, and our results also indicate, that the distribution patterns from different individuals may be diverse and non-uniform.

The reliability of the Kahn technique in our laboratory was controlled by comparisons of all positive reports with the results of parallel Kahn tests made by the Venereal Disease Research Laboratory. Apart from minor variations in degree of positivity in borderline sera, the reports from the 2 laboratories seldom conflicted.

Every subject who showed any positivity with the repeat Kahn or with any of the battery components was studied clinically by a trained syphilologist, to establish the presence or absence of syphilis.

Terminology. Simple and unconfusing terms have been chosen for classifying the positive reactions. "Positive reactions of questionable reliability" seemed an appropriate designation when sero-positivity was found once only, for the reason that it is objective and non-interpretative. To call such instances "technical false positives," as is often done, would be shutting one's eyes to the possibility that serologically reactive substances may have been transitorily present only at the time of the first test. Furthermore, the term "technical" implies an error on the part of technician or laboratory, whereas, in

actual fact, the hemolysis which gives rise to most extrasomatic positive reactions originates in the physician's venepuncture.

"Non - syphilitic positive reactions" seemed a good descriptive term for the repeated finding of sero-positivity in individuals, when diagnostic study could not establish the existence of syphilis. Such reactions are at times referred to, more euphemistically, as "biologic false" or "non-specific" positives. The criteria for all diagnostic categories are discussed in the ensuing paragraphs.

TABLE 1.—DIAGNOSTIC CLASSIFICATION OF POSITIVE SEROLOGIC REACTIONS (WITH THE STANDARD KAHN TEST) SHOWN BY 82,070 ENROLLEES

Diagnosis	No.	All enrollees (%)	Adequately studied positive reactions (%)
1. Single positives of questionable reliability	257	0.33	*39.9*
2. Non-syphilitic positives	86	0.105	*13.4*
3. Indicative of congenital syphilis	41	0.05	*6.4*
4. Indicative of acquired syphilis	260	0.32	*40.3*
Subtotal of adequately studied cases	*644*	*0.78*	*100.0*
5. Inadequately studied cases (unclassified)	134	0.18	
Total	778	0.95	

Criteria and Results. Of the 82,070 consecutive specimens, 778 (0.95%) were read as positive or doubtful. A tabulation of the clinical diagnoses arrived at after more detailed study is given in Table 1.

1. *Positive Reactions of Questionable Reliability.* Since "a single positive report cannot be considered valid evidence of syphilis or a biologic false positive reaction,"[3] all men whose initial specimens were reported positive and whose repeat tests were all negative, were released from supervision without further follow-up.

The group was comprised of 257 individuals, about 40% of all enrollees adequately studied. Only 31 (roughly 12%) were strong reactors (4+, 3+). The remainder gave weak or partial reactions (2+, doubtful 1+, doubtful ±, negative ±).

2. *Non-syphilitic Positives.* The disposition of sero-positive individuals with no clinical manifestations of syphilis was, in general, that of the Venereal Disease Control Branch of the Army.[17] After 3 months of serologic observations, "men whose serologic reaction reversed to negative are discharged from observations as non-syphilitic. Those who show a persistently positive test are regarded as syphilitic, and all others still showing conflicting serologic reactions are subjected to further observation and study."

In the study of these cases, syphilis and the other diseases known to evoke serologic responses were searched for carefully when recording the history and in the physical examinations. The patients' reactions were followed bi-weekly by serologic battery and quantitative Kahn tests. When the titer remained constant or manifested a rise the disposition was deferred for at least 3 months before the diagnosis of syphilis was seriously entertained. When the titer fell progressively without treatment, in the absence of any other reason to suspect syphilis, the case was classed as non-syphilitic positive reaction. Cerebrospinal fluid specimens were taken when the blood reactions remained persistently positive. The presence of syphilis in the wife, children, or parents could only be investigated occasionally since few trainees had their families with them. Doubtful instances which could not be observed for a full 3 months, and a few in whom the diagnosis was still uncertain after 3 months, have been classed as "inadequately studied."

Of the total series of enrollees, 86 (0.105%) were diagnosed as non-syphilitic positive reactors. This figure represents 13.4% of the group of positive cases adequately studied.

In this series of subjects, the blood specimens for serology were collected prior to the prophylactic inoculations given new arrivals. Therefore, the influence of such

injections in eliciting positive reactions was in effect only with the small minority of trainees who were enlisting in the Maritime Service freshly after release from the Army or Navy.

3. *Congenital Syphilis.* Syphilis was classified as congenital when the subject gave a history of having positive serology tests or receiving specific treatment during early childhood; or with stigmata such as iritis, keratitis or deformed teeth, which were rarely seen. A corroboratory story was often obtained of the parents or other siblings also receiving treatment. No patient was placed in the congenital class if he gave a history or exhibited any clinical evidences of chancre, secondary rash, or other phenomena of acquired disease.

The diagnosis seemed well established in 41 of the full series (0.05%). This represents 6.4% of the well-studied cases.

4. *Acquired Syphilis.* The nomenclature of the Bureau of Social Hygiene of the City of New York[2] was utilized for subclassification:

"A. PRIMARY. Evidence of chancre with or without palpable drainage node. Darkfield positive—serology may be either negative (sero-negative primary) or positive (sero-positive primary).

"B. SECONDARY. Evidence of cutaneous, mucous membrane or mucocutaneous lesions with symptoms of generalization, such as adenopathy, early eye involvement or early central nervous system invasion. Wassermann positive.

"C. EARLY LATENT. (Asymptomatic.) Positive serology and no detectable clinical signs or symptoms—infection of less than 4 years duration, with or without treatment. If duration is unknown, patient less than 25 years of age.

"D. LATE LATENT. (Asymptomatic.) Positive serology and no detectable clinical signs or symptoms. Infection of 4 or more years duration, with or without treatment. If duration is unknown, patient 25 years of age or more.

"E. LATE. Demonstrable clinical signs or symptoms of syphilis of cardiovascular, cerebrospinal, cutaneous, osseous, or other organs and tissues. Confirmatory serology, spinal fluid, or Roentgen examinations."

A total of 260 cases of acquired syphilis were recognized, representing 40.3% of the group of cases adequately studied. The incidence of the various stages is listed in Table 2. More than half of the cases, 149 of the 260, were in the latent stage.

5. *Inadequately Studied.* This category comprises all men who left the training station before a reasonably exact diagnosis could be made. The chief factor responsible for the largeness of this group was the sudden mustering out of trainees which would take place whenever the need for more men by the merchant marine suddenly became pressing. In such urgencies a weakly positive Kahn titer was not deemed a cogent cause to detain a man from being shipped out. These men were advised to report to a physician on return from sea. So far as is known, no individual voluntarily disenrolled because of a positive serology finding.

TABLE 2.—AGE GROUP DISTRIBUTION OF ACQUIRED SYPHILIS CASES

Age groups (yrs.)	No. enrollees	Primary (No.)	Secondary (No.)	Early, latent		Late, latent		Late		Total	
				No.	%	No.	%	No.	%	No.	%
16–20	50,437	0	1	52	0.10	5	0.01	3	0.01	61	0.12
21–25	11,950	0	1	34	0.28	7	0.06	2	0.02	44	0.40
26–30	11,930	0	0	37	0.31	23	0.19	3	0.03	63	0.54
31–35	5,419	0	0	21	0.39	23	0.42	3	0.06	47	0.89
36 and over	2,334	0	0	5	0.21	35	1.50	3	0.21	45	1.90
Totals	82,070	0	2	149		93		16		260	
% of all 82,070 enrollees		0.0	0.002		0.18		0.11		0.22		0.32

The membership of the unclassified group was found to possess the same age group distribution and the same ratio of weakly to strongly positive reactions,

within the range of normally expected variation, as did the total series of cases adequately studied. It is a reasonable presumption, therefore, that for this unclassified group the diagnostic breakdown was essentially the same as for the series adequately studied. The absolute figures thus far given for the various diagnostic sub-groups may accordingly be augmented by $\frac{1}{6}\frac{3}{4}\frac{4}{4}$ (20.6%) in order to have a more accurate picture of the frequency of the various diagnostic categories within the total population of enrollees.

Comments. *The Age Factor.* Tables 2 and 3 list the incidence of the several conditions discussed in this paper, arranged according to age group class intervals.

The 86 non-syphilitic positive reactors exhibited no significant change from age group to age group, which suggests that, as a general principle, the biochemical influences which give rise to transitorily positive responses are active independently of the age factor in adult males.

In congenital syphilis the age groups also show a distribution which appears to be entirely random (Table 3). This to-be-expected relationship vouches for the accuracy of the diagnoses made.

Acquired syphilis, in contrast, increased with ascending age. This relationship held for the sub-groups as well as for the total group. As for the 1 apparent exception—early latent syphilis in the men 36 years and older—statistical computation shows that the decline in prevalence lies within the possibility of chance occurrence.

Inasmuch as the Selective Service statistics which have been or will be compiled do not include ages 16 and 17, the years of late adolescence, more detailed data for the age group 16 to 20 years are presented (Table 4). The non-syphilitic positive reactions show no significant change with relation to year of age. Acquired syphilis on the other hand was almost non-existent at 16 years but rose sharply with the succeeding years.

Serologic Patterns of False Positive Reactions. Table 5 is a comparison of reactivity of the 7 member tests of the battery to positive non-syphilitic sera.

In considering this comparison, one must keep in mind that the sero-positivity of these individuals was detected originally by the Kahn Standard test, in other specimens collected 2 days to 3 months earlier. Positive Kahn reactions, as expected, had the highest prevalence. Positive Mazzini reactions were the next most numerous. The Kolmer complement fixation test excited less positive reactions than did any of the flocculation tests. A similar lack of "false" reactivity with the Kolmer test had been noted by Reim

TABLE 3.—COMPARATIVE DISTRIBUTIONS OF NON-SYPHILITIC POSITIVE REACTORS AND OF SYPHILIS CASES IN RELATION TO AGE GROUPS

Age groups (yrs.)	No. enrollees	Non-syphilitic positives		Congenital syphilis		Acquired syphilis		Total syphilis	
		No.	%	No.	%	No.	%	No.	%
16–20	50,437	57	0.11	27	0.05	61	0.12	88	0.17
21–25	11,950	11	0.09	9	0.08	44	0.37	53	0.44
26–30	11,930	11	0.09	3	0.03	63	0.53	66	0.55
31–35	5,419	6	0.11	1	0.02	47	0.87	48	8.90
36 and over	2,334	1	0.04	1	0.04	45	1.93	46	1.97
Totals	82,070	86	0.10	41	0.05	260	0.32	301	0.37

TABLE 4.—COMPARATIVE DISTRIBUTION OF NON-SYPHILITIC POSITIVE REACTORS AND OF CASES OF ACQUIRED SYPHILIS WITHIN THE AGE GROUP 16 TO 20 YEARS

Age (yrs.)	No. enrollees	Acquired syphilis		Non-syphilitic positives	
		No.	%	No.	%
16	12,084	2	0.02	7	0.06
17	16,686	13	0.08	21	0.13
18	14,859	22	0.14	24	0.16
19	3,803	14	0.40	3	0.08
20	3,005	10	0.33	2	0.07

and Elsberg[13] when they surveyed, with a 6-test battery, soldiers whose sera had become transitorily positive in the course of vaccinia, primary atypical pneumonia, and upper respiratory tract infections. These authors commented that in leprosy and malaria, by contrast, the complement fixation tests may demonstrate a higher prevalence of sero-positivity than will the flocculation tests.

proportion of the positive reactions encountered in the initial test may have been positive, not because of extraneous causes, but because their sera at the time of the first test contained reactive substances which had faded away by the time of the second test. If we assume that these statistical influences are mutually neutralizable, the total prevalence of non-syphilitic positives in our series may

TABLE 5.—COMPARATIVE REACTIVITY OF SERA FROM 73 PATIENTS DIAGNOSED AS "NON-SYPHILITIC POSITIVE" TO STANDARD SEROLOGY TESTS

Name of test*	No. sera tested	Negative		Dbtfl. and weakly positive (2+ and less)		Strongly positive (3+, 4+)	
		No.	%	No.	%	No.	%
Kahn Standard	120	29	24	53	44	38	32
Mazzini Flocculation	119	44	37	50	42	25	21
Kline Exclusion	55	24	43	12	40	9	17
Hinton Flocculation	109	68	62	21	19	20	19
Kline Diagnostic	52	33	63	14	27	5	10
Eagle Flocculation	114	80	70	13	10	21	20
Kolmer Complement Fixation	114	96	84	8	7	10	9

* Arranged in order of decreasing reactivity.
All individuals were detected originally by the Kahn Standard test. Every serum listed here was "positive," if only weakly so, to at least one of this battery of tests. Not every test was always performed on each specimen.

Incidence of Non-syphilitic Positives. It is conceivable that some of the individuals placed in this group had latent syphilis, unknown to themselves, or perhaps were deliberately concealing a history of previous antisyphilitic treatment. The number of such syphilitics erroneously termed non-syphilitic positives can only be presumed. The size of the estimate depends upon one's index of suspicion. However, inasmuch as the prevalence of non-syphilitic positive reactions (as diagnosed) was essentially uniform, regardless of age, in contrast with the prevalence of acquired syphilis, which rose concurrently with advance in age, it may be presumed that the number of unrecognized instances of syphilis mistakenly placed in the non-syphilis group was probably not appreciable.

Some of the trainees who gave positive or doubtful reactions on arrival might have been found negative on later study were it not for the stimulating effect of the multiple inoculations (against typhus, smallpox, typhoid and paratyphoid, tetanus, yellow fever) which were given coincidentally with the follow-up serology tests. Conversely, a certain but unknown

be taken as 13.4% of 778, or 104; approximately 1 in 790 for the total population. This rate is somewhat higher, though of the same order of magnitude, as Eagle's "uncorrected" rate of 1 in 1126 college students[5] and is essentially the same as the prevalence in Stokes' series of 1 in 708 Red Cross donors.[16]

Weak vs. Strong Reactions. The strength of the reactivity was related to the occurrence of syphilis as follows: Of 345 weakly positive specimens only 65 (18.8%) were adjudged due to syphilis. Of 299 strongly positive reactions, 236 (78.9%) were adjudged due to syphilis (Table 6; Fig. 1). To generalize from these data, a weakly or partially positive reaction with the Kahn Standard test means that the probabilities are 4 out of 5 that syphilis cannot be demonstrated by careful study. Conversely, a strongly positive reaction means that the probabilities are 4 out of 5 that a syphilitic infection is clearly present.

Prevalence of Syphilis. The prevalence of sero-positive syphilis in this series was approximately 46.7% of 778, or 363; this gives a rate of 4.4 per 1000 and is ap-

proximately one-tenth of that estimated by Vonderlehr and Usilton[18] for the northeastern United States. Their compilation comprised all males between 21 and 35, whereas 60% of the men here surveyed were of age 16 through 20, when syphilis is at its lowest. Moreover, Negroes formed but a small fraction of our series.

It will be recalled that in Stokes' series only 40.5% of the Red Cross donors with positive reacting bloods were finally judged to have syphilis.

Summary. A survey has been made of the incidence and meaning of 783 positive reactions to the Kahn Standard serologic test for syphilis, encountered in a population of 82,070 consecutively examined

TABLE 6.—DISTRIBUTION OF WEAK AND STRONG SEROLOGIC RESPONSES (TO STANDARD KAHN TEST) WITHIN THE DIFFERENT DIAGNOSTIC GROUPS

	Original Kahn test weakly positive (neg. ±, dbt. ±; dbt. 1+, pos. 2+)		Original Kahn test strongly positive (3+, 4+)	
	No.	%	No.	%
Single positives of questionable reliability	226	88	31	12
Non-syphilitic positives	54	63	32	27
Indicative of congenital syphilis	11	27	30	63
Indicative of acquired syphilis	54	21	206	79
Subtotal of adequately studied cases	*345*	*54*	*299*	*46*
Inadequately studied cases (unclassified)	75	56	59	44
Total	420	54	358	46

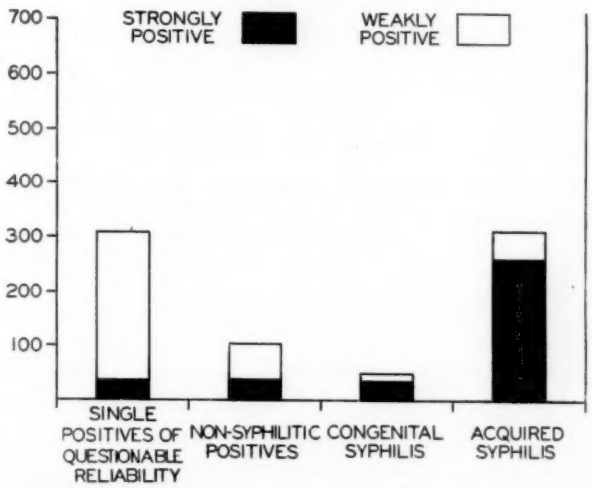

FIG. 1.—Graphic presentation of the distribution of the diagnoses made after clinical study of all positive Kahn reactors encountered among the 82,070 consecutively examined enrollees. "Strongly Positive" refers to those whose first tests were read as 3+ or 4+. "Weakly Positive" comprises all with titers read as 2+ or lower.

Another reason for the difference probably lies in the criteria used for diagnosis. The above authors, and the others cited in the introduction of this paper, based their diagnoses primarily upon the results of serologic tests, whereas in this series the presence or absence of confirmatory findings in history and physical examination were also taken into consideration.

male Maritime enrollees aged 16 to 54 years.

Diagnostic study of the men with positive sera indicated (in round numbers): 40% had single positive reactions of questionable authenticity, 13% had non-syphilitic persistently positive reactions, 7% had congenital syphilis, and 40% had acquired syphilis. The rates for non-

syphilitic positive reactions and for congenital syphilis were essentially constant with all age groups. The rate for acquired syphilis increased progressively with advance in years.

Thus, in one laboratory's experience, the findings of a positive or doubtful response to a Kahn Standard serologic test led to the diagnosis of syphilis in 47%, or less than half, of a group of healthy American men. Weakly positive reactions proved to mean syphilis in only 20% of the group, whereas strongly positive reactions proved to mean syphilis in 80% of the group.

The majority of patients with positive serology here described were studied and diagnosed by Dr. John J. Kobes, Surg. (R), U. S. P. H. S. The serologic battery was performed under the direction of Dr. J. F. Mahoney, Medical Director, U. S. P. H. S. I am indebted to both of these energetic syphilologists for making their records available, and also to Dr. John Fertig, Professor of Biostatistics, Columbia University, for critical appraisal of the statistical comments in this paper.

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INTESTINAL PARASITES DIAGNOSED AT AN ARMY GENERAL HOSPITAL IN THE SOUTH PACIFIC

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With the onset of the war in the Pacific area, an increase in intestinal parasitic infection had been expected by medical practitioners, parasitologists, and other laboratory workers. Over a period of 2 years, a large number of patients, who have had duty in various islands in the South Pacific, have been admitted to a general hospital in that area for varied clinical conditions. During the course of their hospitalization, it has been possible to examine a number of these patients for intestinal parasites, and ultimately to analyze the incidence of both protozoan and helminth infections. Further, as a result of this work, it has been possible to compare our results with surveys carried out by various workers[2,3,6,10,12,13] among personnel in different locales in the United States, in South America, and in the South Pacific. Mumford and Mohr[11] have, in a very excellent tabulation, given the type and the distribution of parasites in the islands of the South Pacific. We believe that our analysis may help in keeping this information up to date.

Material and Methods. Our material is based upon stool studies from soldier patients who have spent up to 3 years in the various islands in the South Pacific area. The patients varied both in duration and degree of exposure and in the islands in which they resided. These patients arrived from other medical installations usually without a previous laboratory diagnosis. Examination for intestinal parasites were carried out on these patients because of (*a*) clinical findings suggestive of parasitic infection, (*b*) elevated eosinophil count, (*c*) fever of unknown etiology, (*d*) previous diagnosis of an intestinal parasitic infection; in this final group, some had been given previous therapy.

Stool examinations were carried out by direct smears using both saline and D'Antoni's iodine.[5] It was customary to run a routine iodine stained smear on every stool. Saline smears were made on all fluid stools and at least once for each patient, regardless of the consistency of the stool. In addition to the direct smears, a simplified procedure of the Faust concentration method[4] was carried out routinely. A suspension of a small particle of a stool made in zinc sulfate (sp. gr. 1.180) was filtered through several layers of gauze into a shell vial. This vial was filled to the top with zinc sulfate. A coverslip was then placed over the top of the vial and permitted to remain in contact with the zinc sulfate for 45 minutes. It was then removed and placed on a slide and examined with the aid of the iodine stain. In addition to the above routine procedures, permanent smears were obtained in cases of doubtful identity. These smears were fixed in Schaudinn's fluid and stained with Heidenhain's hematoxylin. A further technique utilized was a modification of the Baermann procedure[1] for the detection of helminth larvæ. This technique was employed in suspected cases of Strongyloides. A large particle of the stool was placed in a small wire meshed basket lined with gauze. This was then placed in a funnel and a volume of water added so that the entire stool specimen was just submerged. After incubation for 12 to 24 hours, 10 ml. was drawn off, centrifuged, and examined for larvæ. In special instances, the swab method of van Hofe[14] was used for the detection of *Enterobius vermicularis*. Proctoscopic swabs were obtained at the discretion of the ward officer and sent to the laboratory for examination. Direct saline smears were always made from these specimens and examined immediately for trophozoites.

Results. (*a*) *Protozoa.* This series involves a total of 4323 stool examinations on

1114 patients, with an average of 3.8 examinations per patient. Table 1 reveals the incidence obtained in this survey. Our results for *Endamœba histolytica* (4.9%) are comparable with those of Boeck and Stiles for the United States and indicate only a slightly higher incidence of amebiasis. This figure is definitely lower than the value of 8.09% obtained by Markell[10] for *E. histolytica*. The results on the incidence of the other protozoans compare favorably with those of Markell. Low incidence for Giardia may be explained by the fact that many of these patients have received atabrine, which is an effective therapeutic agent in giardiasis (Love and Taylor[7]). We should like to point out that there is a low incidence for the other intestinal flagellates. It may be possible that these forms, like Giardia, are susceptible to atabrine.

(*b*) *Helminths.* The incidence of helminth infections is based on a series of 3415 patients. In this group, a total of 11,358 stool examinations were carried out with an average of 3.3 stool examinations per patient. The data (Table 2) indicate a definitely higher incidence of hookworm infection as compared with the reports from the United States. We obtained an incidence of 13.2% for hookworm as compared with 3.9% obtained in the survey of Boeck and Stiles. Furthermore, our results are higher than those of Markell's[10] survey from the South Pacific and slightly lower than Romeu's figure of 14.6% for *Necator americanus* in a tropical area of Brazil. In this study hookworm infections are as frequent in soldiers from the northern states as from the southern states. Consequently, we feel that the increased parasitism was acquired in the South Pacific. In 3 instances, adult hookworms were recovered from the stools and the species was determined. In all 3 cases, it was found to be *Ancylostoma duodenale*.

TABLE 1.—INCIDENCE OF INTESTINAL PROTOZOA OF MAN

Author:	Boeck and Stiles	Hopp	Rothman and Laskey	Burrows	Romeu	Markell	Present study
Locale:	U. S. A.	Indiana	Phila.	So. Car.	Brazil	So. Pacific	So. Pacific
No. patients examined:	8029	771	306	2055	2500	1371	1114
Protozoa	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Endamœba histolytica	4.1	3.1	3.6	1.7	10.4	8.09	4.90
Endamœba coli	19.6	61.9	17.3	16.3	...	13.66	25.10
Endolimax nana	13.2	31.0	12.7	12.4	...	22.50	19.40
Iodamœba bütschlii	5.0	3.8	0.7	0.3	...	3.72	4.10
Dientamœba fragilis	...	...	2.3	...	...	1.60	
Giardia lamblia	6.5	1.9	10.0	1.9	10.0	1.96	1.50
Chilomastix mesnili	3.1	8.8	0.7	3.3	...	0.32	0.28
Trichomonas hominis	...	0.1	...	3.3	...	0.40	1.40
Enteromonas hominis	...	...	0.3	...	...	0.16	1.20
Embadomonas intestinalis	...	...	...	...	...	...	0.35
Isospora hominis	...	...	...	...	...	0.37	0.35

The coccidian, *Isopora hominis*, was found in 4 cases of this series. The pathogenicity of this organism is still questionable (Magath[9]). Markell[10] points out that some 200 cases have been reported in man thus far. He found 5 additional cases in his series, of which only 1 showed effects of parasitism, namely dysentery. In the present series 2 patients presented *I. hominis* as a single infection. Of these 2, 1 had a mild diarrhea and enterocolitis. The other case presented no intestinal disturbances. In all instances the oöcysts were found present in the stools on 3 to 5 examinations, thus ruling out the possibility of the presence of this organism as a contaminant.

During routine microscopic examinations of many specimens of gastric fluid and bile, several cases harboring parasites were discovered. Two were found with *Trichomonas hominis*, 2 with *Enteromonas hominis*, and 1 with *Embadomonas intestinalis*. One biliary drainage was obtained showing tremendous numbers of *Giardia lamblia*.

Heterodera radicicola (*marioni*) is a common plant parasite. According to Craig and Faust,[4] this nematode has been reported in man, but there is no evidence that it is a true parasite in the human. In the present series, 6 cases were obtained. In all instances, with 1 exception, *H. radicicola* was associated with multiple parasitic infection. Clinical findings were not associated with the presence of this organism.

the zinc sulfate flotation method on all stools, only 65.8% were positive at the first examination, 88.4% were positive at the third examination and 95.1% were positive at the fifth examination.

The data in Table 4 are self-explanatory and indicates the number of multiple infections.

One instance of intestinal myiasis was obtained. The fly larvæ were recovered from the stools and identified as Sarcophaga sp., which has already been described in the literature as one of the etiologic agents of intestinal myiasis (Lyon and Mizelle[8]).

TABLE 2.—INCIDENCE OF INTESTINAL HELMINTHS OF MAN

Author:	Boeck and Stiles	Hopp	Burrows	Romeu	Markell	Present study
Locale:	U. S. A.	Indiana	So. Car.	Brazil	So. Pacific	So. Pacific
No. patients examined:	8029	771	2055	2500	1371	3415
Helminths	(%)	(%)	(%)	(%)	(%)	(%)
Hookworm	3.9	...	4.5	14.6	8.46	13.20
Trichuris trichiura	2.1	0.3	0.8	20.5	2.12	2.40
Strongyloides stercoralis	0.1	3.2	0.8	8.6	1.38	1.50
Ascaris lumbricoides	1.1	...	1.4	19.5	0.37	0.67
Enterobius vermicularis	0.8	7.4	0.5	...	0.07	0.41
Hymenolepis nana	0.5	0.1	0.5	...	0.07	0.23
Schistosoma mansonia	...	...	...	4.0		
Heterodera radicicola	...	...	...	...	...	0.18
Tænia saginata	...	...	...	...	0.07	
Tænia sp.	...	...	...	...	...	0.03

TABLE 3.—ANALYSIS OF THE NUMBER OF EXAMINATIONS REQUIRED FOR A POSITIVE LABORATORY DIAGNOSIS OF 165 PATIENTS INFECTED WITH HOOKWORM

No. examinations	Increase in No. new positives with each examination	Total No. positive	Positive diagnosed with successive stool examinations (%)
1	126	126	76.3
2	22	148	89.6
3	9	157	95.1
4	5	162	98.1
5	2	164	99.3
6	1	165	100.0

A single case of tæniasis was discovered, but further identification was impossible as no segments were recovered. In 1 case many larvæ of *Strongyloides stercoralis* were obtained during biliary drainage.

A question that interested us was to determine the number of examinations required before a negative diagnosis may be accepted. For this study, we decided to use the hookworm data (Table 3). In a series of 165 patients, 76.3% were positive with only 1 examination, while 3 examinations gave 95.1% positive. After 5 examinations, 99.3% were positive. In a similar survey on an earlier group of men, during which time we were unable to use

Summary. 1. A survey was made for intestinal parasites in soldier patients (15,681 examinations) from an Army General Hospital located in the South Pacific area.

2. The incidence of protozoan infection in 1114 patients is 4.9% with *Endamœba histolytica;* 25.1% with *Endamœba coli;* 19.4% with *Endolimax nana;* 4.1% with *Iodamœba bütschlii;* 1.5% with *Giardia lamblia;* 1.4% with *Trichomonas hominis;* 1.2% with *Enteromonas hominis;* 0.28%

TABLE 4.—MULTIPLE PARASITIC INFECTIONS

Double Infections	
E. coli and *E. nana*	34
E. coli and hookworm	21
E. coli and *E. histolytica*	9
E. coli and *I. bütschlii*	7
E. coli and *T. trichiura*	5
E. coli and *S. stercoralis*	2
E. coli and *H. radicicola*	1
E. coli and *C. mesnili*	1
E. coli and *G. lamblia*	1
E. nana and hookworm	10
E. nana and *E. histolytica*	6
E. nana and *T. trichiura*	5
E. nana and *I. bütschlii*	4
E. nana and *T. hominis*	2
E. nana and *E. hominis*	2
E. nana and *G. lamblia*	1
E. nana and *S. stercoralis*	1
I. bütschlii and hookworm	4
I. bütschlii and *E. histolytica*	3
I. bütschlii and *G. lamblia*	1
I. bütschlii and *E. hominis*	1
E. histolytica and hookworm	2
E. histolytica and *C. mesnili*	1
E. histolytica and *E. hominis*	1
E. histolytica and *G. lamblia*	1
G. lamblia and hookworm	1
T. hominis and hookworm	3
T. hominis and *E. vermicularis*	1
T. trichiura and hookworm	3
T. trichiura and *S. stercoralis*	2
T. trichiura and *H. radicicola*	1
T. trichiura and *A. lumbricoides*	1
Hookworm and *S. stercoralis*	3
Hookworm and *H. radicicola*	1
Triple Infections	
E. coli, *E. nana* and hookworm	7
E. coli, *E. nana* and *E. histolytica*	6
E. coli, *E. nana* and *I. bütschlii*	5
E. coli, *E. nana* and *C. mesnili*	1
E. coli, *E. nana* and *T. hominis*	1
E. coli, *E. nana* and *T. trichiura*	1
E. coli, *I. bütschlii* and *E. histolytica*	2
E. coli, *S. stercoralis* and hookworm	2
E. coli, *E. histolytica* and hookworm	2
E. coli, *E. histolytica* and *C. mesnili*	1
E. coli, *E. histolytica* and *E. hominis*	1
E. coli, *I. bütschlii* and *S. stercoralis*	1
E. coli, *E. histolytica* and *H. radicicola*	1
E. coli, *T. trichiura* and hookworm	1
E. coli, *I. hominis* and *T. trichiura*	1
E. coli, *H. nana* and hookworm	1
E. coli, *H. radicicola* and hookworm	1
E. nana, *T. trichiura* and hookworm	3
E. nana, *S. stercoralis* and hookworm	3
E. nana, *T. hominis* and hookworm	1
E. nana, *T. hominis* and *G. lamblia*	1
S. stercoralis, *T. trichiura* and hookworm	1
Quadruple Infections	
E. coli, *E. histolytica*, *S. stercoralis* and hookworm	2
E. coli, *E. histolytica*, *E. nana* and hookworm	1
E. coli, *E. histolytica*, *E. nana* and *I. bütschlii*	1
E. coli, *E. histolytica*, *S. stercoralis* and *T. trichiura*	1
E. coli, *E. nana*, *G. lamblia* and *H. radicicola*	1
E. coli, *I. bütschlii*, *E. hominis* and *T. trichiura*	1
E. coli, *S. stercoralis*, *E. intestinalis* and hookworm	1
E. nana, *I. bütschlii*, *I. hominis* and hookworm	1

with *Chilomastix mesnili;* 0.35% with *Isospora hominis;* and 0.35% with *Embadomonas intestinalis.*

3. The incidence of helminth infection in 3,415 patients is 13.2% with hookworm; 2.4% with *Trichuris trichiura;* 1.5% with *Strongyloides stercoralis;* 0.67% with *Ascaris lumbricoides;* 0.41% with *Enterobius vermicularis;* 0.25% with *Hymenolepis nana;* 0.18% with *Heterodera radicicola;* and 0.03% with Tænia sp.

4. The incidence of hookworm in the present survey is higher than those recorded on the surveys from the United States.

5. Recovery of adult hookworm in 3 cases showed the species to be *Ancylostoma duodenale.*

6. Analysis of the data on 165 cases of hookworm showed that 76.3% were positive after 1 examination; 95.1% were positive after 3 examinations and 99.3% after 5 examinations.

7. It is suggested that atabrine prophylaxis commonly used in this area explains not only the low incidence of Giardia, but also the low incidence of the other intestinal flagellates.

8. Four new cases of *Isospora hominis* are noted.

9. One new case of intestinal myiasis, due to Sarcophaga sp. is reported.

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EFFECT OF GRADED DOSES OF THYROXIN ON EXPERIMENTAL GOITERS, INDUCED BY PROMIZOLE*

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The thyroid stimulating effect of the more recently identified iodine resistant goitrogens is thought to be due to the inability of the thyroid gland to synthesize thyroxine at levels adequate to maintain normal metabolism.[1] This reduction of available thyroid hormone presumably permits an increase of the elaboration of thyrotropic hormone by the anterior lobe of the pituitary, resulting in the production of the goiters. Thyroxin, when provided parenterally during the time the goitrogen is given, will completely prevent the thyroid changes. Three to 10 μg. daily prevented thyroid changes in animals fed a Brassica seed diet.[13] Goiters produced by sulfaguanidine[10] were entirely prevented by giving thyroxin at a level of 1 μg. for each 10 gm. of body weight. Thyroid hyperplasia was abolished by the addition of 1% of thyroid powder to the diet of animals which were given sulfaguanidine[2] and was effectively prevented by giving 5 μg. of thyroxin daily to animals which were given thiouracil.[4] Between 2 and 3 μg. of thyroxin daily prevented thyroid enlargement in both male and female chicks receiving 0.1% of thiouracil in their diet for 14 days.[12] Changes similar to those seen in cretinism, which were induced in young rats given thiouracil, were prevented by administration of thyroxin.[9] Metabolic rates were returned to normal in animals receiving thiouracil by the daily injection of 4.75 μg. of d,l-thyroxin, while 4.8 μg. were required to return the weights of the thyroid glands to normal levels.[14]

Promizole, a sulfone shown to exert a favorable influence on experimental tuberculosis, is likewise goitrogenic.[6,8] The thyroid hyperplasia observed in animals given promizole could not be prevented by giving large amounts of iodine but was completely controlled by giving thyroxin. Furthermore, the hyperplasia of the thyroid gland did not occur in animals, previously hypophysectomized, when given the goitrogen.[7] Data suggest the conclusion that promizole, like the thiourea derivatives, exerts its influence by inhibiting the synthesis of thyroxine resulting in an increased elaboration of thyrotropic hormone by the anterior lobe of the pituitary.

This report covers the results of a study undertaken to determine the amount of thyroxin necessary to prevent the changes of the thyroid gland which promizole will induce in animals.

Method. Forty young male rats having an average weight of 65 gm. were selected for this study. They were arranged into 8 groups of 5 rats each. Five were fed the standard ration (Friskies) without the goitrogen, constituting Control Group 1. Five ate the ration to which promizole had been added at a level of 0.5% and constituted Control Group 2. The animals of the 6 remaining groups ate the diet containing promizole but were given, intraperitoneally, solutions of d,l-thyroxin† containing the following daily amounts: 1, 2, 4, 6, 8 and 10 μg. respectively. The experiment continued for 28 days.

At the end of this test period the oxygen consumption of each animal was recorded

* Promizole (4,2′-diaminophenyl-5′-thiazolyl sulfone) was made available for this study through the courtesy of Drs. E. A. Sharp and L. A. Sweet of Parke, Davis & Co.

† Made available for our use through the courtesy of Dr. E. C. Kendall, Mayo Clinic, Rochester, Minnesota.

and the average metabolic rate was computed in calories per square meter per hour, using the method previously described.[3] Samples of blood were taken from the heart of each animal and, using standardized pipets, the total red and white cell counts were determined. Using the Van Allen hematocrit tubes, red cell volumes in cubic microns were determined and the grams of hemoglobin per 100 cc. of blood were read on the Cenco-Sheard-Sanford hemoglobinometer. Differential distributions of the leukocytes were determined from smears appropriately stained.

The thyroid and pituitary glands were removed from etherized animals, weighed on a precision balance to 0.1 mg. and fixed in formalin. Thyroids were sectioned at 3 microns and stained with hematoxylin and eosin. Using an oil immersion lens (× 90) and a 6 × ocular with an echelon micrometer scale, the heights in microns of 200 thyroid acinar cells were measured for each animal in Control Group 1 and 100 acinar cells were measured for Control Group 2 and for each test animal which had received thyroxin. The means with their probable errors were computed.

Results. The daily administration of thyroxin to the promizole fed rats did not counteract the toxic effects of the drug, so as to induce increased or normal rates of growth (Table 1).

The data on the average oxygen consumption of the animals are condensed into Table 2 and graphically shown in Figure 1. Promizole (Control Group 2) decreased the metabolic rate during the 28 days, 19.7% below that of the normal Control Group 1. Two μg. of thyroxin daily kept the metabolic rate 10.4% higher than that of Control Group 2 but 11.3% below the normal Control Group 1. Four μg. of thyroxin daily maintained an oxygen consumption equal to that observed in animals of Control Group 1. Six μg. daily raised the rate significantly, but only slightly so; while 10 μg. per day induced a real increase of the amounts of oxygen consumed (Table 2).

Daily administration of thyroxin to rats eating the promizole ration, lessened the extent of hyperplasia of the thyroid,

TABLE 1.—THYROID AND PITUITARY GLAND WEIGHTS OF ALL ANIMALS AT THE END OF THE EXPERIMENT

Group*	Attained body wt. (gm.)	Thyroxin daily (μg.)	Thyroid wt. (mg.)		Pituitary wt. (mg.)	
			Absolute	Per 100 gm. body weight	Absolute	Per 100 gm. body weight
Control 1	145.6	0	9.2±0.4†	6.3±0.3	5.1±0.3	3.5±0.1
Control 2	91.0	0	30.5±2.0	33.5±1.0	4.8±0.3	5.2±0.3
Test 1	97.6	1	17.5±2.2	17.7±1.9	3.4±0.2	3.5±0.1
Test 2	76.8	2	9.1±0.4	11.8±0.3	2.4±0.1	3.1±0.1
Test 3	80.1	4	7.2±0.2	9.0±0.4	2.6±0.1	3.2±0.1
Test 4	83.0	6	8.7±0.5	10.1±0.4	3.2±0.2	3.8±0.2
Test 5	89.2	8	6.9±0.3	7.6±0.1	3.0±0.3	3.4±0.1
Test 6	86.0	10	7.7±0.3	8.9±0.3	2.8±0.2	3.2±0.3

* Each group contained 5 rats. † Probable error of the mean.

TABLE 2.—MEAN OXYGEN CONSUMPTION

Group	Thyroxin daily (μg.)	Mean oxygen consumption (calories per sq. m. per hr.)	Difference	
			Calories	%
Control 1	0	44.2±0.7*		
Control 2	0	35.5±1.3	−8.7±1.5†	−19.7
Test 2	2	39.2±1.3	3.7±1.8‡	10.4
Test 3	4	46.1±1.8	10.6±2.2‡	29.9
Test 4	6	56.5±2.5	21.0±2.8‡	59.2
Test 5	8	50.8±0.6	15.3±1.4‡	43.1
Test 6	10	58.9±0.7	23.4±1.5‡	65.9

* Probable error of the mean.
† Control Group 2 − Control Group 1.
‡ Respective test group − Control Group 2.

as had been anticipated (Table 1). Promizole in the diet (Control Group 2) produced goiters with a relative weight more than 5 times, and an absolute weight more than 3 times that of the thyroids in the normal Control Group 1. One μg. of thyroxin daily restricted the thyroid hyperplasia to about a half, and 2 μg. animals (Table 1) were of interest in that the administration of the thyroid hormone did result in maintaining smaller pituitaries in promizole fed rats. Even in small amounts thyroxin appeared to inhibit pituitary changes so that both absolute and relative weights were significantly less than the average pituitary weights in

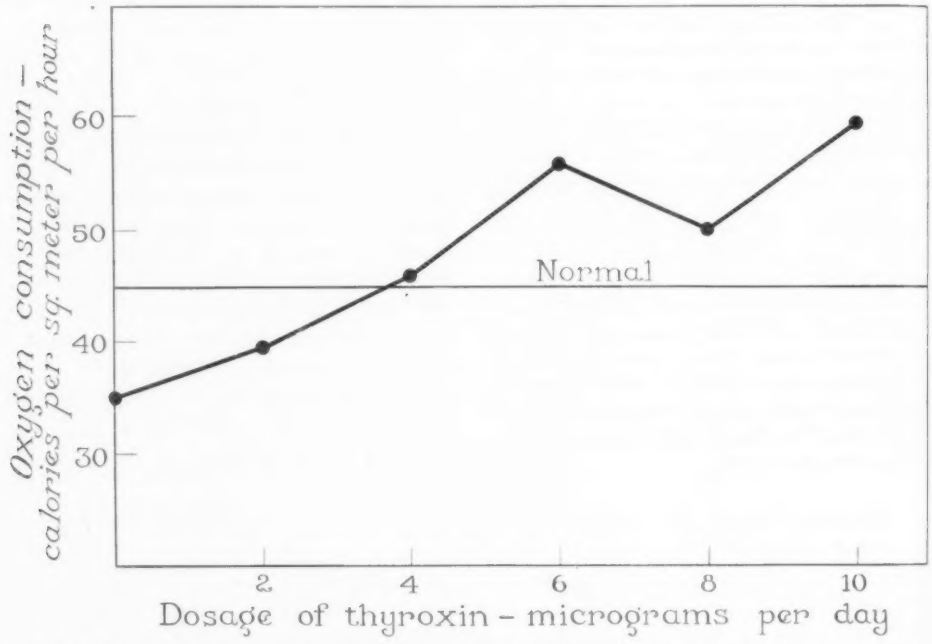

FIG. 1.—Changes in calories per square meter per hour recorded at the end of the experiment. Normal data were obtained from Control Group 1.

to about a third of that observed in animals not given supplementary hormone. Four μg. of thyroxin daily reduced the weights of the thyroids considerably below the average weight in the normal control animals. Larger amounts did not further reduce the average thyroid weights. Mere shreds or residues of colloid remained in acini of thyroids which had been subjected to the influence of promizole (Fig. 2). Thyroxin counteracted this effect. One μg. per day greatly lessened these changes in the colloid patterns and 2 μg. per day completely nullified them (Fig. 3).

Both the absolute and the relative weights of the pituitary glands of these animals which received the goitrogen but were not given the hormone.

The measurements of the thyroid acinar cell heights have been condensed into Table 3. The percentage distributions of these data within 4 of the 8 groups are shown in Figure 4. According to Figure 5, a daily amount of the hormone equal to about 1.75 μg. was sufficient to prevent any increase in size of the cells comprising the thyroid epithelium.

The data assembled from heart samples of blood are condensed into Tables 4 and 5. Promizole induced a slight anemia, with lowered red cell counts and increased red cell volumes, but the hemoglobin levels were not significantly reduced. This

standard ration provides very much more protection to animals receiving promizole than do purified rations fortified by the better known vitamin B fractions. The average total leukocyte count, however, was greatly reduced from control levels in all animals given promizole.

Although there were trends to suggest that thyroxin may have had some effect in counteracting the destructive influence of promizole on the red cells, yet the data are not too impressive. Certainly the hemoglobin levels were not significantly altered by administration of thyroxin and the leukopenia observed to accompany the feeding of promizole in this experiment

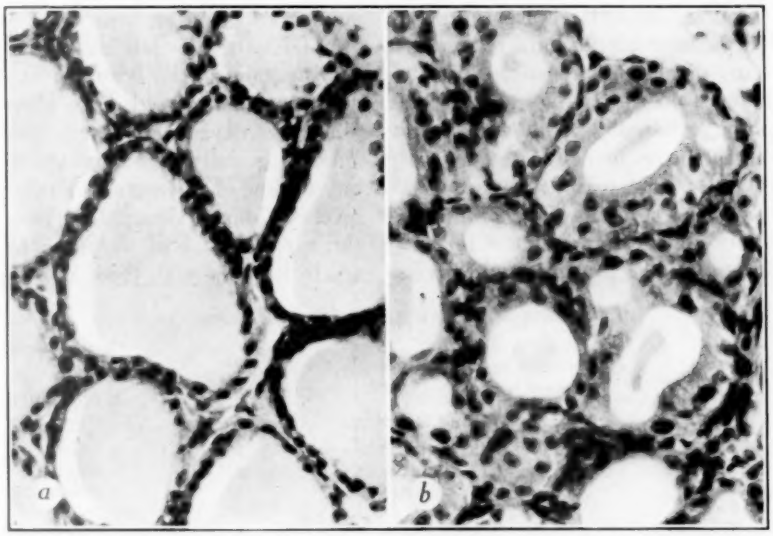

FIG. 2.—Histologic appearance of thyroid glands. *a*, Control Group 1, which did not receive promizole. *b*, Control Group 2, which received promizole in the diet at a level of 0.5% in ration.

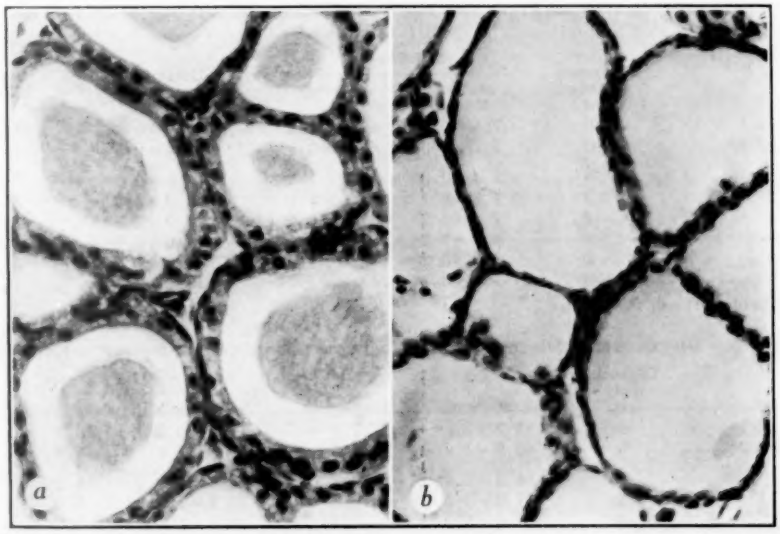

FIG. 3.—Histologic appearance of thyroid glands. *a*, Test Group 1, which received promizole and 1 μg. of thyroxin daily. *b*, Test Group 2, which received promizole and 2 μg. of thyroxin daily.

was not corrected by giving thyroxin (Table 4). But a study of the differential distribution data showed a reaction of the neutrophilic granulocytes to thyroxin that would seem to warrant further study (Table 5). Significant elevations were observed to occur in the percentages of granulocytes in the blood of all animals which received 2, 6 or 8 μg. of the hormone daily. Among animals receiving 10 μg. daily, the scatter of the data rendered a satisfactory deduction impossible. Significant changes in the monocytes or the eosinophilic or basophilic granulocytes did not occur in the hormone treated animals.

Comment. The toxic reactions sustained by young animals given promizole, particularly anorexia and failure to grow normally, were not prevented by giving thyroxin. Thiouracil, given subcutaneously,[9] inhibited the growth of young rats but when thyroxin was given the normal growth rates were restored. Since thyroxin was without effect on the growth of our promizole fed animals, retardation of development was obviously not due to a deficiency of thyroid hormone.

The decline of the rate of oxygen consumption, however, was the result of a thyroid hormone deficiency, for the administration of the hormone at the level of 2 μg. daily exerted some effect on the amount of oxygen consumed. Slightly less than 4 μg. daily was adequate to prevent any decline of the metabolic rate from the level of Control Group 1. These data on the rapid decline of oxygen consumption are in keeping with those observed when

TABLE 3.—THYROID ACINAR CELL HEIGHTS

Group	Diet	Thyroxin daily (μg.)	Thyroid acinar cell height (μ)
Control 1	Standard	0	4.61±0.04*
Control 2	0.5% promizole	0	11.90±0.10
Test 1	0.5% promizole	1	8.00±0.10
Test 2	0.5% promizole	2	3.10±0.02
Test 3	0.5% promizole	4	2.70±0.02
Test 4	0.5% promizole	6	2.70±0.02
Test 5	0.5% promizole	8	2.80±0.03
Test 6	0.5% promizole	10	2.70±0.03

* Probable error of the mean.

TABLE 4.—BLOOD VALUES OBTAINED FROM SAMPLES OF HEART BLOOD AT THE END OF THE EXPERIMENT

Group	Thyroxin daily (μg.)	Erythrocytes		Hemoglobin (gm. per 100 cc. of blood)	Leukocytes (thous. per c.mm.)
		Mill. per c.mm.)	Volumes (c.μ)		
Control 1	0	7.8±0.1*	49.9±1.2	14.0±0.3	15.3±0.7
Control 2	0	6.7±0.3	60.1±0.1	13.1±0.5	5.9±0.4
Test 1	1	7.4±0.2	59.9±1.3	14.5±0.5	7.5±0.8
Test 2	2	7.3±0.2	55.0±1.1	13.3±0.2	5.4±0.6
Test 3	4	7.4±0.2	51.0±1.1	11.9±0.5	3.7±0.3
Test 4	6	6.9±0.2	57.4±2.4	11.9±0.6	7.2±0.8
Test 5	8	7.9±0.2	48.9±1.3	12.6±0.5	5.5±1.9
Test 6	10	7.5±0.2	54.2±0.7	13.8±0.2	6.4±0.6

* Probable error of the mean.

TABLE 5.—DIFFERENTIAL DISTRIBUTION OF LEUKOCYTES AT THE END OF THE EXPERIMENT

Group	Thyroxin daily (μg.)	Lymphocytes	Monocytes	Granulocytes		
				Neutrophilic	Eosinophilic	Basophilic
Control 1	0	85.7±1.3*	2.1	11.7±0.4	0.3	0.2
Control 2	0	82.0±1.4	1.5	14.2±1.4	1.8	0.5
Test 1	1	84.2±1.4	0.9	13.2±1.0	1.2	0.5
Test 2†	2	77.0±3.5	0.8	21.2±1.9	0.6	0.4
Test 4	6	64.0±5.2	2.1	30.6±2.5	0.7	2.6
Test 5	8	63.0±1.4	1.5	35.0±2.4	0.5	0
Test 6	10	74.0±3.1	1.0	23.5±10.6	1.0	0.5

* Probable error of the mean. † Data on Test Group 3 not obtained.

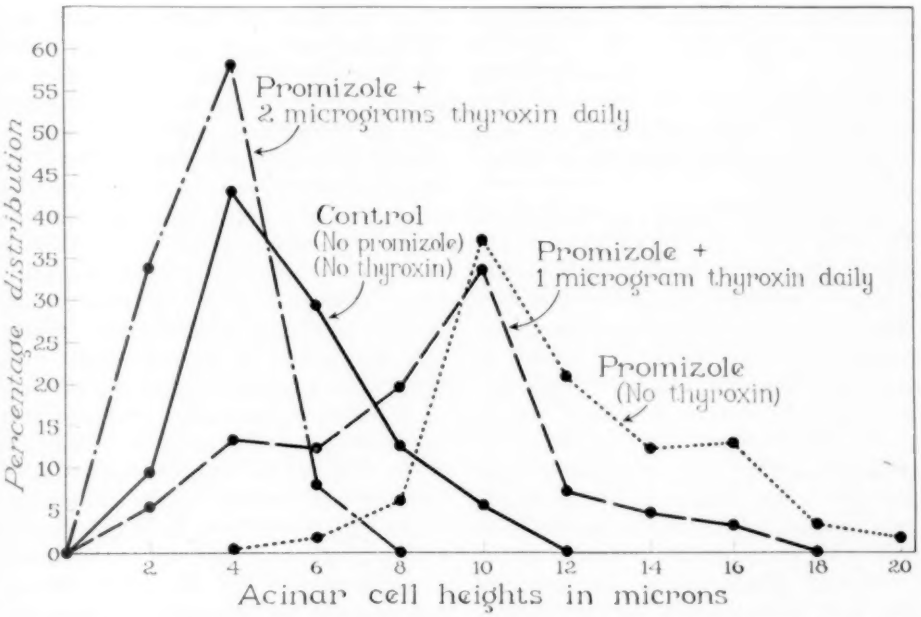

FIG. 4.—Percentage distributions of the heights of the thyroid acinar cells in 4 groups of animals: Control Groups 1 and 2; Test Groups 1 and 2.

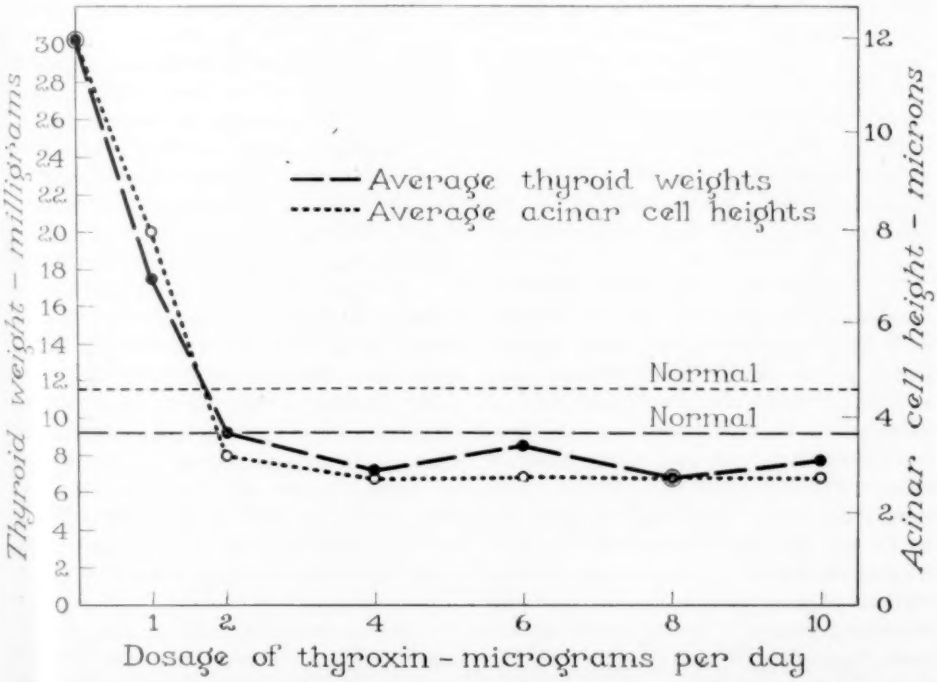

FIG. 5.—Changes in the weights of the thyroid glands and the heights of the thyroid acinar cells in animals which received promizole and in those which received promizole together with graded doses of thyroxin daily. Normal data were obtained from Control Group 1.

sulfaguanidine was given.[11] Reineke, Mixner and Turner[14] lowered the metabolic rate 23.7% when thiouracil was provided in the drinking water at a level of 0.1% for 14 days. These rates were returned to normal by the daily injection of 4.75 μg. of d,l-thyroxin.

The protection afforded by thyroxin to the colloid bodies of animals given promizole is of interest. Promizole, like other goitrogens, produces changes in the metabolism of the thyroid gland resulting in the atrophy or destruction of its colloid reserves. Whereas 1 μg. of thyroid hormone appeared to provide some protection to the colloid bodies, 2 μg. provided complete protection. The homogeneous appearance of the colloid was retained; its staining qualities were preserved. The reasons for the retention of normal colloid in thyroid glands of animals receiving promizole when provided adequate blood thyroxin levels are not clear. Since the basal metabolism of such animals was increased when the hormone was given, the thyroid cell, in the presence of an adequate or even increased level of blood thyroxin, may retain its selective function for metabolizing iodine and synthesizing thyroxin. There is need, it would seem, for a study of the capacity of the hyperplastic thyroid to take up radio-iodine in the presence of normal or increased blood levels of thyroxin.

We learned that the blood levels of promizole were progressively lower in animals into which thyroid hormone was injected at the levels which ranged from 1 to 8 μg. The average concentrations of promizole in the blood stream of the Control Group 2 animals and in the blood of those receiving 1 μg. of thyroxin daily were statistically alike, ranging from 6.6 to 15 mg. per 100 cc. of blood (average 9.2 mg. per 100 cc.). The average concentrations of promizole in the blood of the remaining groups of animals were as follows: 2 μg. of thyroxin, 8.2 mg. per 100 cc.; 4 μg., 7.5 mg. per 100 cc.; 6 μg., 5.8 mg. per 100 cc.; 8 μg., 3.7 mg. per 100 cc.; and 10 μg., 6.3 mg. per 100 cc. Increased rates of metabolism induced by the added daily amounts of thyroxin may have facilitated the more rapid destruction or elimination of the drug and may serve to explain the absence of the colloid damage.

A more probable explanation for the retention of normal colloid bodies in rats treated with thyroxin and fed promizole is that in the presence of an adequate blood thyroxin level no demands were made on the colloid for thyroid hormone. The colloid bodies thus remained unexhausted and their usual thyroxin reserve remained intact. It is evident that 1 μg. daily was insufficient to meet the body requirements, so that some demands were made on these colloid reserves. The inability of the thyroid cells to replace them resulted in the atrophic colloid patterns When 2 μg. were given daily, the bodily requirements were met and there were no demands made on the thyroid for thyroid hormone, so that normal colloid patterns persisted. The conclusion may well be drawn that the requirement of thyroxin by rats of this age, as used in this experiment, was about 2 μg. per day.

In our experience with animals to which promizole was fed or into which it was injected, real increases of the weights of the pituitary glands occurred during the production of the goiter. This fact may be correlated with an increased elaboration of the thyroid stimulating hormone. Observations elsewhere have contributed to the conclusion that increases of the relative numbers of basophilic cells did occur in the pituitaries of animals made goitrous by sulfaguanidine.[10] On the other hand, changes of the microscopic structure of pituitaries of animals made goitrous by thiouracil were not observed.[15] Pituitaries of animals made goitrous by a rapeseed diet, however, were reported to contain an increased number of basophilic cells with degranulation of the acidophilic cells.[5]

Whether or not the increased relative weights of the pituitaries of our animals made goitrous by promizole were related to changes of the content or the output of thyroid stimulating hormone, such increased weights did not occur when thyroxin was administered. Even 1 μg. of thyroxin

per day maintained relative weights and larger amounts of the hormone actually reduced the absolute weights of the pituitary glands. The decline of pituitary gland weights was probably dependent on the size and the extent of hyperplasia in the developing goiter. When normal thyroid gland weights and normal histologic patterns were maintained by the injection of 2 μg. of thyroid hormone daily, the weights of the pituitary glands were likewise reduced. Accordingly, 2 μg. of thyroxin per day, or perhaps an amount slightly less, will maintain in rats eating a standard diet containing 0.5% of promizole for 28 days, a normal thyroid gland, with normal or subnormal acinar cell heights, and normal appearing colloid bodies, together with pituitary glands of normal or subnormal weights.

Conclusions. 1. The administration of d,l-thyroxin, in amounts ranging from 1 to 10 μg. daily, to young rats during a period of 28 days, while they were fed a diet containing the goitrogen, promizole, at a level of 0.5%, did not inhibit the usual untoward effects which promizole normally exerts on the growth, appearance and appetite of immature animals.

2. As hitherto shown, promizole depressed thyroid activity. In this experiment the average metabolic rate of animals given promizole in their diet for 28 days was lowered 19.7% from that recorded for the normal control group. Two μg. of d,l-thyroxin daily increased the average rate 10.4% above that of the control group which received promizole alone, while 4 μg. maintained an average oxygen consumption equal to that recorded for the animals which did not receive either thyroxin or promizole.

3. Promizole exerts a thyroid-stimulating influence through the pituitary gland, which, in this experiment, resulted in the development of goiters weighing more than 3 times the average weight of the thyroid gland in control animals. The administration of 1 μg. of d,l-thyroxin daily resulted in the development of goiters which were but slightly less than twice the normal size, while 2 μg. daily resulted in maintaining thyroid glands of normal weights.

4. The heights of the thyroid acinar cells were maintained at normal or less than normal levels by giving 2 μg. of d,l-thyroxin daily. Without any supplemental thyroxin, the average acinar cell height was more than twice that of the normal gland, while 1 μg. of thyroxin was adequate to maintain an average cell height considerably less than twice that of the normal thyroid gland.

5. The changes observed in the colloid of thyroid acini which were induced by promizole were partially prevented by 1 μg. of thyroxin per day and were completely nullified by giving 2 μg. daily.

6. Promizole appears to exert its goiter stimulating effect by preventing the synthesis of thyroxin by the thyroid cell, thereby permitting an increased elaboration of thyroid stimulating hormone by the anterior lobe. By the administration of small amounts of thyroxin daily during the time the goitrogen was provided, thereby maintaining adequate blood levels of thyroxin, normal thyroid glands were maintained.

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NOTE ON HIPPURIC ACID SYNTHESIS IN SENILITY

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In a previous communication (Stern, Hinds and Askonas[5]), it was demonstrated that hippuric acid synthesis is impaired in higher age. The original purpose of that investigation was to determine the detoxifying capacity of the aging organism, particularly during psychoses of the higher age group. There is a striking similarity between certain senile psychotic episodes and delirious psychoses produced by exogenous toxins during other periods of life. On the basis of this clinical fact, it had been assumed that the capacity to detoxify certain metabolites is reduced in old age.

Only hippuric acid synthesis was studied, because it is the only test which has been sufficiently evaluated quantitatively for clinical purposes. This test was found to be markedly impaired in the group of patients examined. Of patients of 60 years or more, 80% were shown to excrete less than 0.85 gm.; the 5 cases diagnosed as senile dementia ranged from 0.18 to 0.7 gm. This impairment was very marked compared with observations on mentally and physically normal old people (Rafsky and Newman[3]).

However, the test is an index of 2 functions: the conjugating power of the liver and the capacity to furnish glycine (by synthesis or by mobilization). Therefore an attempt was made to determine as to which one of these factors was impaired in the aged. This was done by the administration of glycine during the test. A similar procedure has been carried out before in patients with liver diseases (Probstein and Londe[1]) by the oral sodium benzoate test.

Subjects. Twelve patients with senile or pre-senile conditions ranging from the age of 58 to 92 were examined. As controls 6 healthy adults were used and 4 adults of faulty dietary habits. One of the latter was a woman of 56 who suffered from a peculiar anxiety state with anorexia. NPN, blood cholesterol, blood sugar and, in the senile cases, the albumen/globulin ratio were determined in order to exclude cases with gross metabolic disorders.

Method. A test was carried out on 3 consecutive days. On the 1st and 3rd day Quick's intravenous hippuric acid test was carried out in the usual manner, with 1.77 gm. of sodium benzoate. On the 2nd day glycine was added in the following way.

The subject was given 1 gm. of glycine as a 10% watery solution ½ hour before the injection of sodium benzoate. One gm. of glycine was given orally at the time of the injection; ½ hour after this another gram of glycine was administered. Before the injection and 1 hour following it the urine was collected. In the old patients this was done under the supervision of physician and nurse, and in some cases by catheterization. Each subject was given 2 tumblers of water before the injection to assure a sufficient output of urine. Hippuric acid was determined according to Quick.[2]

Results. The results are presented in 2 graphs and in Table 1.

The 6 normal control cases show that the hippuric acid values on the 1st and 3rd day correspond to the normal cases given by Quick and do not differ more from one another than 0.2 gm. An additional oral administration of glycine on the 2nd day increased the hippuric acid output by 0.14 to 0.28 gm. in all but 1 case.

Ten out of 12 senile cases showed a low hippuric acid synthesis to begin with. In 9 out of these 10 the hippuric acid output was raised by glycine feeding; in 5 cases by 0.3 gm. or more, *i. e.*, more than had been seen in the normal control subjects. In 6 out of these 9 the increase led to a normal level of hippuric acid excretion (0.85 gm. or higher).

In the second line of Figure 2 there are 4 control cases of individuals with faulty dietary habits. All suffer from anorexia of a psychologic origin, one was a textbook case of anorexia nervosa. One of these patients showed a finding unique among the entire investigated group, *i. e.*, a depression of the hippuric acid excretion during glycine administration.

Discussion. From these experiments it is obvious that in most senile cases the impairment of hippuric acid synthesis is only partly or not at all due to faulty conjugation. Otherwise we could not explain why exogenous glycine could improve the mechanism. In fact, 2 cases with conspicuously low values were raised to normal; this is particularly interesting because in animal experiments with N isotopes exogenous glycine, even when fed in excess, supplied only one-third of the hippuric acid glycine (Rittenberg and Schoenheimer[4]). However, it was not possible to explain what factor determines the various patients' individual patterns. An attempt was made to correlate the difference between the glycine-free days and the 2nd day with various data such as the patient's weight-height index, dynamometer readings, and diet history; but no such correlation could be found.

However, although no correlation could be found with the patients' diet history, the phenomenon seems nevertheless to reflect on the nutritional state; for we saw a similar pattern in those otherwise healthy young individuals with anorexia (Fig. 2, Case SC and McC). In fact, the increase produced by glycine feeding in those cases is remarkable. Two values were raised by 0.67 and by 0.71 gm. respectively. Among these cases, however, there was one with a peculiar inverted reaction; in this case (CAN) the hippuric acid excretion was depressed with the administration of glycine. Errors in the collection or determination were excluded. It is impossible to explain this phenomenon but it is noteworthy that the same patient,

TABLE 1.—HIPPURIC ACID VALUES

No.	Name	Sex	Age	Wt. (lb.)	Ht.	Diagnosis	Average 1st + 3rd test of hippuric acid (gm.)	Deviation between 1st + 3rd test (gm.)	Hippuric acid (gm.) excretion following glycine administration	Increase (gm.)
1	HI	M	28	186	6′3″	Normal	1.39	±0.01	1.30	−0.09
2	ME	F	30	120	5′4″	Normal	1.04	±0.03	1.32	+0.28
3	BO	M	29	200	6′1″	Normal	1.40	±0.05	1.62	+0.22
4	RO	F	26	130	5′4″	Normal	1.10	±0.10	1.36	+0.26
5	NO	M	27	168	6′	Normal	1.10	±0.02	1.36	+0.26
6	CR	M	24	150	6′	Normal	1.25	±0.01	1.39	+0.14
7	SC	F	29	85	5′1″	Anorexia nervosa	0.81	±0.09	1.52	+0.71
8	McC	F	56	86	5′1″	Anxiety state with anorexia	0.83	±0.01	1.50	+0.67
9	CAN	F	33	92	5′2″	Agitated depression	1.40	±0.01	1.10	−0.30
10	MU	F	38	93½	5′1″	Obsessive neurosis with anorexia	0.93	±0.03	1.16	+0.23
11	HOF	F	84	142	5′2″	Paranoid psychosis	0.80	±0.03	1.04	+0.24
12	CA	F	74	99	5′3″	Senile dementia paranoid	0.37	±0.07	0.84	+0.47
13	GRA	F	76	121	4′7″	Manic-depressive	1.01	±0.02	0.98	−0.03
14	GR	F	81	94	4′10″	Senile dementia	0.70	±0.02	1.06	+0.36
15	HO	F	58	101	5′½″	Pre-senile dementia	0.83	±0.08	1.20	+0.37
16	JOH	F	92	110	5′2″	Old schizophrenia with superimposed senile process	0.51	±0.11	0.85	+0.34
17	JO	F	64	107	5′	Senile dementia	0.43	±0.02	0.59	+0.16
18	LE	F	59	115	5′3″	Pre-senile dementia	0.91	±0.09	1.02	+0.11
19	MA	F	58	98	5′4″	Senile dementia	0.25	±0.05	0.49	+0.24
20	SH	F	80	109	4′11″	Senile dementia	0.57	±0.10	0.68	+0.11
21	SM	F	75	105	5′6″	Schizophrenia with superimposed senile processes	0.40	±0.05	0.60	+0.20
22	CO	F	68	98	5′5″	Senile dementia	0.43	±0.03	1.03	+0.60

a lean asthenic woman who had been a very poor eater for some time, was gaining 6 pounds during the week in which the test was carried out. Even if we assume hypothetically that her N metabolism was in an anabolic phase during this period it is difficult to explain why glycine added to the diet should not only not be used for conjugation but even depress that function. This would be contrary to the results of experiments with sodium benzoate feeding of growing animals

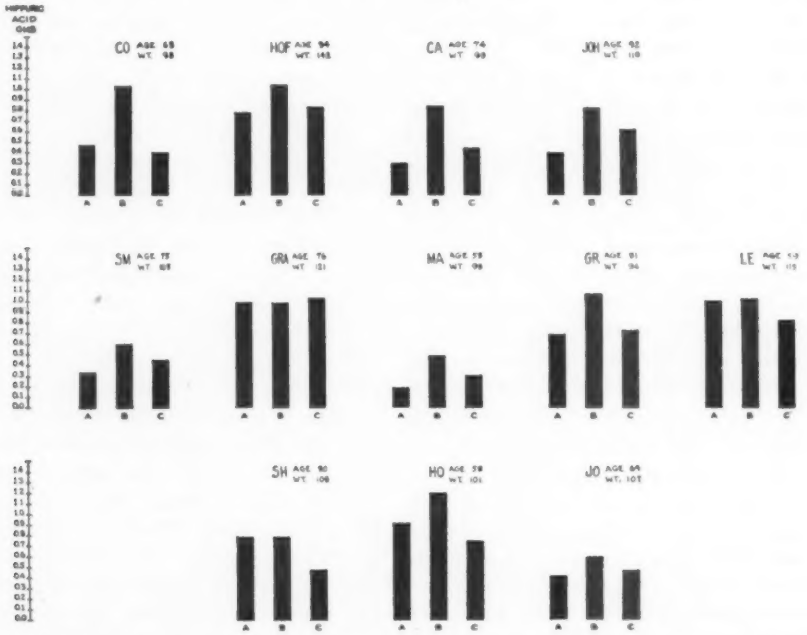

FIG. 1.—Senile and presenile cases. The hippuric acid excretion (in gm.) on 3 consecutive days is represented by 3 columns. *A* = 1st day, *B* = 2nd day, *C* = 3rd day. On the 2nd day (*B*) glycine was administered orally before and during the test (see test).

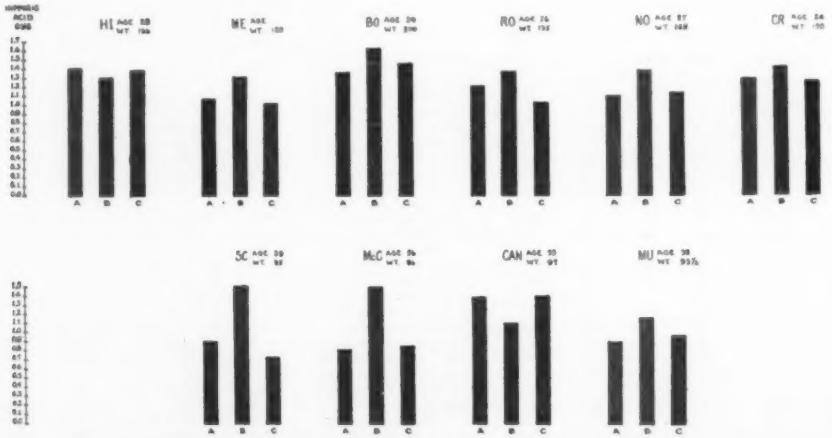

FIG. 2.—Control cases. The graphic presentation is the same as in Figure 1. The cases on the upper line are healthy young people. The cases on the lower line are patients with anorexia of psychologic origin, and debility, but otherwise healthy.

(White[6]). Probstein and Londe[1] found similar cases among their patients with impaired liver function, and attempted to explain this seemingly paradoxical finding by various hypotheses on glycine metabolism.

Summary. Hippuric acid synthesis was studied in 12 senile patients on 3 consecutive days; on the 2nd day glycine was administered orally before and during Quick's intravenous test. Six healthy adults and 4 adults with anorexia of psychologic origin served as control cases.

Ten of 12 senile cases showed an abnormally low hippuric acid synthesis to begin with. In 9 out of these 10, hippuric acid synthesis was increased to a varying degree by glycine administration. From this finding it can be concluded that faulty hippuric acid synthesis in old age is mainly due to an impairment of the organism to furnish glycine rather than to an impairment of the mechanism of conjugation. The difference in the patterns of these cases could not be correlated to any known clinical feature.

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THE ACTIVITY OF PENICILLIN IN THE INTRANASAL PNEUMOCOCCUS INFECTION OF MICE*

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The method of infection of mice by inhalation had been thoroughly studied by Stillman and his co-workers[12] in the years 1924–1931, and excellent investigations as to the pathology and pathogenesis of the experimental pneumonia were contributed by Webster and Clow,[14] and Rake.[7] From these studies might be seen that the natural resistance of mice towards the intranasal infection is considerable and that neither all strains of pneumococci nor all breeds of mice were suitable for infections of this type. It was probably the finding of Stillman and Branch[13] that treatment with high doses of alcohol was apt to break the natural and acquired resistance which induced Neufeld and Etinger-Tulczynska[6] to adopt the method of short ether anesthesia preceding the nasal infection. By this modification, very consistent and reliable infections could be obtained.

The reason why the intranasal infection with pneumococci and the subsequent pneumonia of mice was not more widely used in the determination of antipneumococcal activity might probably be found in the fact that satisfactory infections require suitable strains of pneumococci and a susceptible breed of mice; another reason might be that most of the known chemotherapeutic agents exert only a very poor activity in this infection. We were unable to find any reference as to the activity of sulfonamides in the pneumonia of mice; our own experience, covering roughly 10 years during which the intranasal infection was routinely studied with all agents which exerted antipneumococcal activity towards intra-abdominal infection of mice, showed clearly that in the group of sulfonamides, only exceptionally was a satisfactory activity found. Even in these cases, the therapeutic effect was generally not more than prolongation of life dependent on the duration of the treatment. Final survivors were observed merely occasionally.

From the observations of Gross and Cooper,[2] Kepl and Gunn,[3,4] Raiziss, Kolmer and Rule,[8] and Loughlin, Bennett, Flanagan and Spitz,[5] who used the intratracheal and intrabronchial pneumococcus infection of rats, may be seen that the pneumonia of these animals responded to a certain degree to treatment with different sulfonamides and sulfones. Wood[16] described particularly good results with sulfapyridine alone, but most of the other investigators[3,4,5] agree that the combination with antipneumococcus serum enhances considerably the activity of the chemotherapeutic agents.

It seemed from these observations that the slow and predominantly bacteriostatic activity of the sulfonamides was not always sufficient to control the pulmonary infections of mice and rats. The different

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mechanism of activity and the more powerful antibacterial action of penicillin gave reason to expect a more definite effect in pulmonary infections.

Methods. Two strains of pneumococci were used in our experiments. One was a pneumococcus Type 1, obtained from the School of Hygiene of the University of Toronto. The strain came originally from the laboratories of the Rockefeller Institute. The second strain (Pn. 6302) was a Type 2 strain, obtained from the American Type Culture Collection. Both strains were kept on blood agar and in serum broth and were passed through mice regularly.

The mode of infection was simple: white mice* of 18 to 22 gm. were exposed to ether for 2 minutes, and then their noses were dipped into undiluted overnight serum broth of the pneumococci.

Untreated mice died within 30 to 48 hours after the infection. Pneumococci could be detected in the lungs by culturing on blood agar immediately after the infection. After 1 to 2 hours, a heavy infection of the blood was present. Pathologic changes, mostly dark red patches of bronchopneumonic infiltration, were generally not present before 24 hours; but at the time of death, both lungs were hepatized, of dark red color, and filled with exudate.

Treatment with penicillin was administered 1 hour after the infection. In some instances, a single treatment was given. No more than 4 treatments were found necessary, at least not with the doses selected for these experiments. The penicillin used in these studies was sodium salt of penicillin prepared in the Roche research laboratories, its potency as determined by the cup test method was 500 to 800 u./mg.

The succumbed mice, treated as well as untreated ones, were autopsied, and cultures of the lungs and heart blood were taken. Surviving animals were under observation for a period of 3 weeks. From time to time, surviving mice were sacrificed and their lungs examined histologically.

Experimental. It is known by the work of Rake, McKee and their co-workers[9] that very small doses of intra-abdominally injected penicillin were sufficient to control an intra-abdominal infection with pneumococci. Welch, Chandler, Davis and Price,[15] on the other hand, found that an average of 478 u. injected intra-abdominally were required in order to protect 50% of mice infected with 10,000 m.l.d. of pneumococci. We consider 50 to 60 u./20 gm. as the minimal active subcutaneous dose for the control of an intra-abdominal infection with 1000 m.l.d. of pneumococci Type 1, Type 2 and Type 3.[1] Infections with Type 1 pneumococci sometimes responded to a total dose of 40 u./20 gm.

For the treatment of intranasal infections, higher doses were required.

In Table 1, the results of the subcutaneous treatment of intranasal infection with Type 1 and Type 2 pneumococci are given. A total dose of 800 to 1000 u./20 gm. can be considered the minimal active dose giving 50% survivors in the Type 1 infection, while about 1800 to 2000 u./20 gm. were required in infections with the Type 2 pneumococcus. Smaller doses, *e. g.*, 200 to 500 u./20 gm., showed still

TABLE 1.—ACTIVITY OF SUBCUTANEOUS TREATMENT WITH PENICILLIN IN PNEUMOCOCCUS PNEUMONIA CAUSED BY TYPE 1 AND TYPE 2 PNEUMOCOCCI

Total dose (u./20 gm.)	Type 1			Type 2		
	No. mice	No. survivors	% survivors	No. mice	No. survivors	% survivors
25–50	12	1	8.3			
100–150	12	3	25.0			
200	20	8	40.0			
400–500	24	10	41.0	15	0	0
800–1000	24	12	50.0	30	2	6.6
1600	13	9	69.0	5	0	0
1800–2000	..	..	..	60	27	45.0
3000–4000	5	5	100.0	19	15	79.0
Controls	40	1	2.5	75	2	2.6

* Strain of Tumblebrook Farms, Brant Lake, New York.

an appreciable activity in Type 1 infections, but the results with these doses were not always consistent.

The data for Type 1 infections represent the results of single treatment with high doses as well as those obtained by 2 to 4 times repeated treatment with smaller doses. As a rule, higher activity was observed with the latter method. In Type 2 infections, only repeated treatment was given.

In an attempt to analyze these observations, mice were killed 1, 3, 5, 9 and 25 hours following intranasal infection and a single treatment with 1000 u./20 gm. Cultures taken from the lungs showed that the number of pneumococci diminished gradually; after 9 hours, only a few scattered colonies could be recovered. After 25 hours, however, heavy growth from the lungs was again obtained in the cultures. If, however, 2 treatments of 1000 u./20 gm. were given with an interval of 4 hours, the lungs were sterile after 9 hours and stayed sterile if examined 24 hours after the treatment. This finding might explain why a single treatment, though it might be successful in some instances, was generally not sufficient; for the sterilization of the lungs, at least 2 treatments were necessary.

Pathologic findings in surviving treated mice were generally absent. During the first 3 or 4 days after the infection, very small marginal patches of pneumonic infiltration were occasionally found; but later, the lungs appeared normal by gross and microscopic examination.

Similar results were obtained when penicillin was given orally. Table 2 is meant to demonstrate the comparison of subcutaneous and oral treatment in intranasal infections with pneumococcus Type 2.

In both groups of experiments, the same doses of penicillin were found to be active.

The observation that high total doses of penicillin (about 2000 u./20 gm.) had approximately the same effect whether the drug was given parenterally or orally might appear surprising. Experimental and clinical experience suggested that—as a rule—oral treatment required higher doses than the parenteral route. The observations in the intranasal pneumococcus infection were not the only and not the first exception to this rule.[11]

The determination of the penicillin concentration in the blood seemed to offer some explanation for these findings.

The technique used for the determination of the serum level of penicillin was the Rammelkamp method.[10]

Groups of 5 mice were used for every dose and for every interval. The animals were sacrificed after the subcutaneous or oral administration by bleeding them from the axillary artery in ether anesthesia. The blood was pooled; and after clotting, the serum was used for the determination of potency.

The figures in Table 3 represent averages of 2 to 4 determinations with different groups of mice.

From the data on Table 3, it might be seen that in all dosage ranges, the *peak* of the concentration of penicillin in the serum was at least 10 to 20 times higher after subcutaneous than after oral administration. By increasing the subcutaneous dose, a longer duration of comparatively low concentrations was observed; however, the blood level dropped considerably

TABLE 2.—COMPARISON OF SUBCUTANEOUS AND ORAL ADMINISTRATION OF SODIUM PENICILLIN IN INTRANASAL INFECTIONS OF MICE WITH TYPE 2 PNEUMOCOCCI

(2 to 4 Treatments)

Total dose (u./20 gm.)	Subcutaneous			Oral		
	No. mice	No. survivors	% survivors	No. mice	No. survivors	% survivors
800–1000	20	2	10	40	2	5
1800–2000	60	27	45	84	38	45
3200–4000	5	3	60	50	29	58
7200	..	..	..	5	4	80
Controls	125	4	3			

within 2 hours in all dose ranges. The oral administration caused much less spectacular peaks and, accordingly, a more consistent serum level was found for a certain period of time, depending on the dose. Particularly after high doses (1000 u. and more), the serum level after oral treatment was more or less comparable to that of the subcutaneous dose in the later phases (after 2 to 3 hours), and was even occasionally found to be higher, *e. g.*, at the 3 to 5 hour intervals.

Table 3.—Concentration of Penicillin in the Serum of Mice After a Single Subcutaneous and Oral Treatment With Sodium Penicillin

Dose (u./20 gm.)	Route	Interval (minutes) after administration								
		15	20–30	60	90	120	150	180	210	300
100	Subcut.	2.0	1.15	0.25	0.06					
	Per os	..	0.075							
500	Subcut.	25.0	6.60	1.00	0.15	0.015	0.06	0		
	Per os	..	0.38	0.10	0.03	0.02	0.03	0		
1000	Subcut.	22.5	22.50	1.00	..	0.50	0.03	0.03		
	Per os	2.0	0.50	0.08	0.10	0.03	..	0.06	0.015	
2000	Subcut.	22.5	22.50	4.00	..	0.50	0.03	0.03	0	
	Per os	1.0	2.50	0.58	0.13	0.13	..	0.13	0.06	
5000	Subcut.	..	..	128.00	..	..	..	1.00	..	0.06
	Per os	..	..	2.00	..	..	..	1.00	..	1.00

Table 4.—Prophylactic Activity of a Single Dose of Sodium Penicillin After Subcutaneous and After Oral Administration Against Intranasal Infection of Mice With Type 2 Pneumococci (Experimental Pneumonia)

Penicillin (u./20 gm.)	Route of administration	Interval* (hours)	No. mice	Average survival time (days)	% survivors
2500	Subcut.	0	9	4.0	45.0
		1	29	4.3	41.5
		3	27	2.3	22.0
	Per os	0	10	4.0	80.0
		1	30	7.0	40.0
		3	35	5.6	31.0
5000	Subcut.	1	15	3.0	56.0
		3	20	2.3	20.0
		5	15	2.4	14.0
	Per os	1	10	9.0	60.0
		3	20	6.6	25.0
		5	15	7.0	20.0
Controls		..	48	2.0	2.5

* Interval between treatment and infection.

The question arose whether these differences in the blood level were of therapeutic significance. A suitable way of obtaining information to this point seemed the investigation of the prophylactic activity of large single doses of penicillin.

Doses of 2500 and 5000 u./20 gm. were given to mice subcutaneously or orally, and the animals were infected intranasally with Type 2 pneumococci shortly afterwards and after intervals of 1, 3 and 5 hours. The results are given in Table 4.

This table shows that the effect of high doses of penicillin was the same whether the drug was administered subcutaneously or orally. If one considers not only the percentage of final survivors after 3 weeks of observation but also the survival time of the animals which died of pneumococcal pneumonia during this period, it might appear that the oral prophylactic dose was even somewhat more effective.

Similar results were obtained in intra-abdominal streptococcal infections; these experiments will be published in the near future.

Discussion. The intranasal infection of mice with Types 1 and 2 pneumococcus which produced fatal pneumonia and septicemia responded readily to subcutaneous treatment with penicillin. Comparatively high doses, 1000 u. (Type 1) to 2000 u. (Type 2), were required to control the infection in approximately half of the treated animals. Higher doses gave a correspondingly higher percentage of survivors.

The observation that the oral administration of penicillin was as effective as the parenteral one was in agreement with our earlier findings[11] in infections with meningococci, Salmonella and *Borrelia norvi*. In these infections which required high doses of penicillin (200 u. and more), the ratio

$$\frac{\text{Minimal oral dose}}{\text{Minimal subcutaneous dose}}$$

was small and did not exceed values of 1 to 2. On the other hand, in infections which responded to comparatively small doses of penicillin, *e. g.*, the intra-abdominal streptococcal and pneumococcal infections, the ratio was about 10. In more severe infections or in infections with more resistant organisms which required higher doses of penicillin by the parenteral route the ratio oral/subcutaneous dose showed a tendency to drop. The comparison of intra-abdominal pneumococcus infection (ratio 8 to 10^{11}) and intranasal infection (ratio 1) is only one example; the same relation was found in streptococcal infections. While, for instance, the ratio oral/subcutaneous dose was 10 in infections with 1000 m.l.d., it dropped to 1.5 if 1,000,000 m.l.d. of the same streptococcus strain were used for the infection.

The determination of the blood level seemed to offer an explanation for these findings, because in the later intervals after high doses of penicillin, the concentrations of penicillin in the serum were almost identical whether the drug was given per os or subcutaneously. That would mean that the very high initial peak which was only observed after parenteral administration was of lesser significance than the consistent level of comparatively low concentrations to be found in the serum during the hours following the subcutaneous as well as the oral administration.

From the prophylactic and therapeutic experiments in mice, we might draw the conclusion that there was no decisive influence of the peak concentration after high doses of penicillin. The blood level reached after the oral doses was obviously sufficient not only to protect mice against the intranasal infection but also to prevent septicemia and death if the treatment was started 1 hour after the infection.

Whether these findings have some bearing on the penicillin therapy of infections in humans cannot be decided at the present moment because only scanty information as to the dosage and effectiveness of oral penicillin treatment in different types of infections is available. We have, however, the impression that the oral route of penicillin administration based on an adequate dosage schedule might be useful in all conditions where a consistent blood level has to be maintained over a longer period of time.

Summary. 1. Penicillin exerted a very definite effect in the prevention of pneumonia of mice caused by intranasal infection with the pneumococcus Type 1 and Type 2.

2. The minimal active total dose in Type 1 infections was 800 to 1000 units per 20 gm. given subcutaneously in 2 to 4 treatments, while Type 2 required a total dose of 1800 to 2000 u.

3. In intranasal pneumococcus Type 2 infection of mice, oral administration of penicillin was as effective as the subcutaneous route. This was found not only if penicillin was given 1 hour after the infection but also in prophylactic experiments when the mice were infected 1 to 5 hours after the administration of penicillin.

4. The determination of the penicillin level in the serum of mice seemed to suggest that the concentrations obtained after high oral doses were sufficient to control the intranasal pneumococcus infection, although they did not produce as high an initial peak of the serum level as observed after parenteral injection.

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SUCCINYLSULFATHIAZOLE IN THE NUTRITION OF THE RAT

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It has been reported that rats receiving succinylsulfathiazole in *stock* or *natural* diets grow normally and show no evidence of nutritional deficiencies.[8,9] In contrast, the inclusion of succinylsulfathiazole in the usual adequate *highly purified* diets renders such diets incapable of supporting normal growth. The feeding of these diets leads to the development of a variety of deficiency signs including alopecia, achromotrichia, porphyrin-stained whiskers, leukopenia and agranulocytosis.[7,9,12] These outward manifestations of nutritional deficiencies are accompanied by decreased hepatic storage and fecal elimination of folic acid, biotin and even pantothenic acid. The administration of folic acid and biotin will restore such animals to normalcy with respect to both appearance and hepatic vitamin storage.[12]

Although no apparent detrimental effects result from the feeding of succinylsulfathiazole to rats on stock diets, it should be recognized that it is quite possible for an animal to appear normal, but to possess a tissue vitamin storage indicative of a latent or borderline deficiency. Consequently these additional growth studies have included determinations of the hepatic storage and fecal elimination of folic acid, biotin and pantothenic acid when rats were fed succinylsulfathiazole in a complete diet. Fecal *Escherichia coli* counts also have been made as a measure of the bacteriostatic effectiveness of the drug.

Procedure. Thirty-two weanling male albino rats were separated into 2 equivalent groups and were caged individually over wide-mesh screening. They were given food and water *ad libitum*. Group 1 received a diet of 95% powdered "Friskies" and 5% Wilson's 1 to 20 liver powder. Group 2 received a diet composed of 90 parts of the above ration and 10 parts of succinylsulfathiazole. The ratio of succinylsulfathiazole to calories consumed in this diet is approximately 3 times that existing when succinylsulfathiazole is administered in its usual dosage to human patients. On a gm./kilo/day basis the rats ingested 20 to 40 times the recommended human dose. The experiment was of 8 weeks duration and the rats were weighed at weekly intervals. Occasional food intake determinations were carried out on representative rats throughout the experiment. At intervals vitamin determinations also were carried out on complete 24 hour fecal collections. The fecal *E. coli* counts were made on representative rats after they had consumed the diet for 8 weeks. At the conclusion of the experiment all of the animals were killed by decapitation and representative livers were assayed microbiologically for the various B vitamins studied.

The preparation of the fecal and liver samples for microbiologic assay has been described previously.[11] Folic acid was determined microbiologically with *Lactobacillus casei* as the assay organism.[2] Biotin, pantothenic acid and nicotinic acid were determined with *Lactobacillus arabinosus*.[5,6,10] Ten mg. of para-aminobenzoic acid per 100 ml. were included in the assay medium as a sulfonamide inhibitor in the microbiologic assay of feces. A folic acid concentrate was used as a standard in the microbiologic assay of folic acid. The standard has been assayed against a sample of synthetic *Lactobacillus casei* factor, supplied through the courtesy of the Lederle Laboratories, and the results have been recalculated in terms of the presumably pure factor. The *E. coli* counts were obtained by a dilution plate count method using desoxycholate agar as a differential medium.

Results and Discussion. The data obtained have been summarized in Table 1.

The inclusion of 10% succinylsulfathiazole in the adequate non-synthetic diet employed did not alter significantly the growth or normal appearance of the rats. The rats consuming the succinylsulfathiazole-containing diet ingested significantly more food even when the food intakes were corrected for the fact that only 90% of the diet was nutritive material. The average amount of feces produced per day by the rats consuming the sulfonamide diet was a little higher than that produced by the rats on the control diet. The difference was not statistically significant, however, probably because of the great variability in daily feces production that exists in rats.

The fecal elimination of folic acid was significantly depressed in the rats consuming the succinylsulfathiazole diet. Miller has shown, *in vitro*, that sub-bacteriostatic amounts of sulfanilamide markedly decrease the amount of folic acid synthesized by *E. coli*.[3] Probably this phenomenon occurs also with other intestinal organisms and other sulfonamides. In the present experiment the fecal elimination of biotin, pantothenic acid and nicotinic acid was not significantly altered by the administration of succinylsulfathiazole.

The hepatic storage of folic acid was found to be 5 (4.1–6.2) γ/gm. in the control animals and 2.5 (2–3.5) γ/gm. in the sulfonamide-fed group. This difference is statistically significant. Previous studies have shown that the folic acid content of the liver of rats fed *natural* diets may range from about 4–12 γ/gm.[4,12] When rats are reared on *highly purified* diets which furnish no external source of folic acid to the rat, but which are entirely adequate for normal growth, the animals are able, probably by bacterial synthesis in the intestine, to build up an hepatic storage of folic acid in the order of 0.6–1.5 γ/gm. The inclusion of succinylsulfathiazole in the diet reduces this value to 0.2–0.6 γ/gm. and deficiency symptoms

TABLE 1.—SUMMARY OF EXPERIMENTAL DATA

	Control group*	Succinylsulfathiazole-fed group*	P†
Initial weight, gm.	59 (54–66) (16)	60 (56–68) (16)	0.1–0.2
Final weight, gm.	236 (214–276) (16)	231 (188–271) (16)	0.5–0.6
Food intake, gm./day	16.8 (14–20) (36)	20.5 (15–24) (36)	<0.01
Corrected food intake, gm./day	.....	18.5 (13.5–21.6) (36)	<0.01
E. coli organisms in feces, No./gm.	400,000 (128,000–900,000) (3)	270 (20–500) (3)	
Feces produced, gm./day	9.7 (7.6–12.5) (5)	12.7 (8.6–19.7) (6)	0.1–0.2
Fecal elimination of:			
Folic acid, γ/day	31 (26–41) (5)	17 (9–26) (6)	<0.01
Biotin, γ/day	5.3 (3.3–6.7) (5)	4.3 (3.8–4.9) (5)	0.2
Pantothenic acid, γ/day	416 (311–484) (5)	339 (232–430) (6)	0.05–0.1
Nicotinic acid, γ/day	525 (463–576) (3)	507 (474–552) (3)	0.6–0.7
Hepatic storage of:			
Folic acid, γ/gm.	5.0 (4.1–6.2) (8)	2.5 (2.0–3.5) (8)	<0.01
Biotin, γ/gm.	0.93 (0.82–1.0) (8)	0.83 (0.77–0.91) (8)	0.02–0.05
Pantothenic acid, γ/gm.	60 (52–66) (8)	65 (52–75) (8)	0.2

* Accompanying the average values presented are (1) the range in values obtained, and (2) the number of animals involved or determinations performed.

† The P values or significance were calculated by the t test where

$$t = \frac{x_1 - x_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}}$$

ϵ for 30 or more observations $= \dfrac{\Sigma x_i^2 - n\bar{x}^2}{n^2}$

ϵ for less than 30 observations $= \dfrac{\Sigma x_i^2 - n\bar{x}^2}{n\,(n-1)}$

Degrees of freedom $= n_1 + n_2 - 2$

We are indebted to Mr. Joseph L. Ciminera for the statistical calculations.

are encountered. The hepatic storage of folic acid found in the present sulfonamide-fed group was 2 to 4 times that found in rats reared on purified diets and 5 to 10 times that found in rats actually showing evidence of a folic acid deficiency. It should be noted that folic acid is unique among the B vitamins in that the liver storage ordinarily found in stock rats is approximately 20 times the level found in deficient rats. In contrast, pantothenic acid, riboflavin, or biotin-deficient rats have an hepatic storage of the limiting factor that is one-third to one-half that found in stock rats.

Despite the long-continued feeding of succinylsulfathiazole the coliform organisms were essentially absent from the feces at the conclusion of the experiment. Results have been reported in which *E. coli* organisms reëstablished themselves following administration of succinylsulfathiazole for a period of several weeks.[1] The sulfonamide was fed at a level of 0.5% and it is possible that this concentration permitted sulfonamide resistant strains to develop and become reëstablished in the intestine.

In the present experiment leukocyte counts were not made. It has been our experience that low leukocyte counts and agranulocytosis do not occur independent of a depression in growth or a decrease in the hepatic storage of folic acid below a level of about 0.8 γ/gm.

Summary. 1. Succinylsulfathiazole was administered to growing rats over an 8 week period in a dose which, in comparison with the prescribed human dose, was approximately 3 times greater on the basis of % of food intake and 20 to 40 times greater on the basis of gm. per kg. of body weight.

2. The growth, general appearance, hepatic storage of biotin and pantothenic acid and fecal elimination of biotin, pantothenic acid and nicotinic acid were not significantly influenced by the ingestion of the sulfonamide.

3. The animals consuming the sulfonamide-containing diet eliminated significantly less folic acid in the feces and at the conclusion of the experiment were found to have significantly less folic acid stored in the liver. The hepatic storage of folic acid was, however, approximately 3 times that found in rats receiving adequate highly purified diets and 8 to 10 times that found in folic acid deficient animals.

4. The *E. coli* organisms were practically eliminated from the feces of the succinylsulfathiazole-fed rats.

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EFFECT OF CITRATE ON THE REACTIONS OF LEAD WITH BLOOD CELLS AND PLASMA*

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In papers dealing with the use of sodium citrate as an agent in the treatment of plumbism, Kety and Letonoff[8,9] presented evidence that the administration of the citrate results in the prompt reduction of the concentration of lead in the blood and, in most cases, an increase in its excretion. These observations were subsequently confirmed by Cantarow and Trumper.[6] Previously, Aub and co-workers[1] noted a slight increase in the rate of lead excretion following sodium citrate administration.

It has long been known that citrate solutions dissolve certain relatively insoluble lead salts, an action which has been attributed to the formation of a soluble lead citrate complex. Proof of the existence of such a complex in dilute solutions was obtained by Kety,[7] who concluded that even the normal plasma citrate may be responsible for keeping otherwise insoluble lead compounds in solutions and, hence, for their physiologic removal from the blood. This conclusion has been questioned, however, by Bambach, Kehoe and Logan[3] on the basis that lead is carried mainly in the cells.

In view of these observations, it seemed of interest to investigate further the mechanism by which citrate influences lead metabolism. The present paper deals with the effect of citrate on the combination of lead with cells and plasma, as indicated by changes produced in: (*a*) the plasma cell partition; (*b*) the ability of the cells to remove lead from the plasma; and (*c*) the proportion of diffusible lead in the plasma as determined by ultrafiltration. Any process that alters these factors might reasonably be expected to affect the mobilization and excretion of lead.

Methods. All experiments were performed with freshly drawn human blood *in vitro* at a temperature of 37.5° C. (*In vivo* studies will be the subject of a subsequent paper.) Heparin was used as the anticoagulant. Solutions of lead chloride and sodium citrate were added in small amounts to the blood or other fluid under investigation by means of micropipettes, so that the total increase in volume did not exceed 2% of the volume of the specimen.

Lead analyses were made by using radium D, an isotope of lead, as a radio-active indicator and measuring the activity with a Geiger-Müller counter, as previously described.[11] While the values obtained by this method represent added lead only, they roughly approximate the total amounts under the conditions employed in this work, where the quantity of lead initially present was always small in comparison with that added. For the experiments involving the addition of relatively small amounts of lead, blood specimens having an initial concentration of less than 0.01 mg. per 100 cc., as found by the dithizone method, were selected. The concentration of lead in the cells was calculated from the hematocrit value and from the concentrations in whole blood and in plasma, except in the few cases in which the cells, after separation from the plasma, were suspended in saline and analyzed directly.

No attempt was made to determine the initial citrate content of the blood specimens. Normal plasma contains from 1.5 to 4 mg. per 100 cc., according to Schersten,[13] but as much as one-fifth of the amount contained in a sample may disappear within 1 hour.[12] All concentrations given in the present paper refer to added citrate only.

Ultrafiltrates were obtained by means of a filtering system constructed of glass vessels and a membrane of dialyzer cellophane which had been soaked in several changes of distilled water to remove the glycerol. The

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solution and filtrate could come in contact with nothing but glass and the membrane at any point. A positive pressure of about 450 mm. of mercury was employed. With this apparatus, ultrafiltrates of plasma and serum gave negative coagulation tests with heat and acetic acid.

Results. *Plasma Cell Partition.* After preliminary tests, which indicated that in some instances sodium citrate alters the distribution of lead between the plasma and cells, the effect of various concentrations of citrate with different levels of lead was investigated. In the first experiment, graded amounts of the citrate were added to 2 cc. samples of blood. Active lead chloride was then introduced, so as to give a predetermined lead concentration. After standing for 20 minutes with occasional gentle inversion, the mixtures were centrifuged, and samples of plasma were analyzed for lead. Typical results are presented in Figure 1*A*. With a total lead concentration of 0.1 mg. per 100 cc., less than 4% of the lead remained in the plasma, and the addition of citrate had only a slight effect on the amount found in this fraction of the blood. At the level of 0.2 mg. per 100 cc., the proportion of lead in the plasma of untreated blood was greater, but was considerably reduced by the presence of the citrate.

In this connection, it may be pointed out that in clinical and experimental lead poisoning, more than 90% of the element is held by the cells.[3,11] A similar distribution is usually found when soluble lead salts are mixed with blood *in vitro*, provided the concentration is kept comparatively low, *e. g.*, 0.1 mg. per 100 cc. However, the present results show that at higher levels, a higher, but variable, percentage of lead may be found in the plasma, the actual percentage apparently depending upon the manner of mixing, as well as upon individual variations in blood specimens. It is at the higher lead levels that the effect of citrate in facilitating the uptake by the cells becomes most evident.

A blank experiment was performed by repeating the procedure with samples of plasma instead of whole blood. In every case the concentration of lead was the same in the upper and lower portions of the plasma after centrifuging, thus indicating that the disappearance of lead from

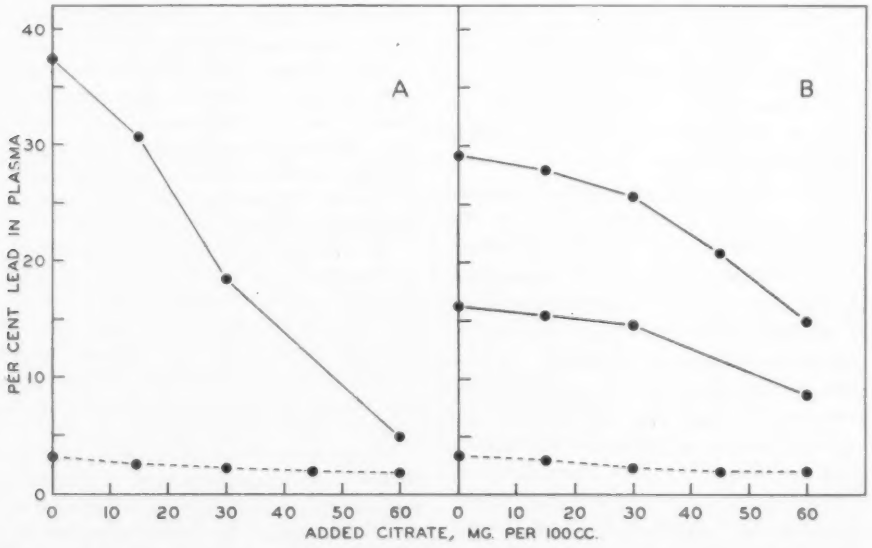

FIG. 1.—The effect of citrate on the distribution of lead between cells and plasma. Solid lines represent a lead concentration of approximately 0.2 mg. per 100 cc ; dotted lines indicate 0.1 mg. per 100 cc. *A*, Lead added to blood containing citrate. *B*, Blood treated with citrate after the addition of lead.

the plasma, with increasing amounts of citrate, was dependent upon reaction with the cells.

Figure 1*B* shows results obtained in the same way as those represented in Figure 1*A*, except that the lead was added to the blood first, and the mixture was allowed to stand for 20 minutes before the introduction of citrate. It will be noted that the citrate produces a smaller effect on the plasma cell partition when added to blood previously mixed with the lead.

The effect of the length of time elapsing between the addition of citrate and centrifugation was also studied. In one experiment in which sodium citrate was added to the blood with a lead concentration of 0.18 mg. per 100 cc., 14 and 5% of the lead was found in the plasma fractions at the end of 5 and 20 minutes, respectively, while in untreated controls the corresponding values were 34 and 32%. Very little change occurred after the first 20 minutes, irrespective of amount of citrate present.

Blood samples were also treated with solutions of certain other substances which have been advocated for use as "deleading agents"—sodium bicarbonate, sodium thiosulfate, potassium iodide, disodium phosphate and ascorbic acid—but in no case was the distribution of lead measurably different from that in untreated controls.

Combination of Cells With Lead in Plasma. Aub and co-workers[2] noted that lead mixed with serum could no longer produce its usual effects on the red cells. As stated by Minot,[10] lead appears to be inactivated by admixture with serum. Bambach, Kehoe and Logan[3] made the further observation that when cells are separated from plasma, and are returned after adding lead to the plasma, they remove considerably less of the element than could be expected from the results of introducing lead solutions directly into whole blood. According to the latter authors, these observations indicate the formation of some type of combination of lead and plasma. In the present work, a study was made of the influence of citrate on the ability of lead to thus combine with plasma.

Blood samples were treated with varying amounts of sodium citrate. The plasma was then separated, and mixed with active lead chloride. After standing for 10 minutes, it was returned to the cells. All samples had a final lead concentration of approximately 0.1 mg. per 100 cc. At the end of 20 more minutes, the mixtures were centrifuged and the two blood fractions analyzed for lead. The results (Table 1) show that under the conditions of these experiments, the presence of added citrate greatly increases the amount of lead transferred to the cells. While 33% remained in the plasma in the absence of citrate, all but 7%, for example, was removed by the cells in presence of 15 mg. of citrate per 100 cc. Thus the ability of the plasma to withhold from the cells, lead previously mixed with it, is greatly reduced by the addition of sufficient citrate.

TABLE 1.—EFFECT OF CITRATE ON THE ABILITY OF CELLS TO REMOVE LEAD FROM PLASMA

Sample	Citrate (mg./100 cc.)	Lead in cells (mg./100 cc.)	Lead in plasma (mg./100 cc.)	% lead in plasma
1	0	0.14	0.065	32
2	3	0.14	0.059	29
3	5	0.15	0.049	24
4	15	0.19	0.014	7
5	30	0.19	0.008	4

The influence of time on this action of citrate is shown in Table 2. Samples of the remixed blood, containing the same amount of lead as before and 15 mg. of citrate per 100 cc., were centrifuged at different periods of time after mixing. Other samples containing no citrate served as controls. As can be seen from the data, lead is taken up rapidly by the cells in the presence of citrate. It will also be

noted, incidentally, that the percentages of lead in the plasma are somewhat higher than the corresponding values obtained in the previous experiment (Table 1). Such variations were encountered when the specimens were collected at different times and from different individuals. Nevertheless, both sets of data clearly show the "activating" effect of citrate on lead mixed with plasma.

Combination of Cells With Lead in Ringer's Solution. In this experiment, washed cells were mixed with lead and citrate dissolved in a special Ringer's solution containing phosphate, as used by Aub and co-workers.[2] All samples had a final lead content of approximately 0.1 mg. per 100 cc. One series of mixtures was centrifuged after 5 minutes, and another at the end of 30 minutes. As shown in Table 3, even a comparatively low concentration of citrate produced a large increase in the amount of lead taken up by the cells and, unexpectedly, higher concentrations proved to be somewhat less effective. In blank tests performed with similar concentrations, no sedimentation of lead occurred in the absence of cells.

TABLE 2.—EFFECT OF TIME ON THE ABILITY OF CELLS TO REMOVE LEAD FROM PLASMA

Time after mixing cells and plasma (min.)	Without citrate			With citrate		
	Lead in cells (mg./100 cc.)	Lead in plasma (mg./100 cc.)	% lead in plasma	Lead in cells (mg./100 cc.)	Lead in plasma (mg./100 cc.)	% lead in plasma
2	0.038	0.16	81	0.15	0.051	26
10	0.067	0.13	67	0.18	0.025	12
30	0.082	0.12	59	0.18	0.020	10
60	0.088	0.11	56	0.18	0.019	9

Transfer of Lead From Cells to Plasma. The reversibility of this reaction was also investigated. Results reported previously[11] showed that lead passes readily from cells to plasma in experimental animals. On the other hand, Blumberg and Scott[4] failed to remove appreciable quantities of lead by washing cells twice with isotonic saline. In the present work, 3 samples of blood containing 0, 5 and 15 mg. of citrate per 100 cc., respectively, were allowed to stand until the cells had reached equilibrium with lead in the plasma. The cells were then centrifuged down, separated as completely as possible from the plasma, and washed 3 times for 5 minutes with an equal volume of fresh plasma containing no added lead. Analyses made on the plasma washings and on the washed cells revealed that the second and third washings had each removed from 2 to 3% of the lead, which was almost as much as could be expected from the results of distribution studies[11] on blood having a similar lead content (0.1 mg. per 100 cc.). The percentages of lead removed from the 3 samples were the same, however, within the limits of experimental error. It may be concluded, therefore, that the transfer of lead from the cells to the plasma is not appreciably affected by the presence of extra citrate.

Ultrafiltration. Plasma and serum samples were treated, in turn, with sodium citrate and lead chloride, and allowed to stand a few minutes, after which, the mixtures (containing 0.1 mg. of lead per 100 cc.) were subjected to ultrafiltration. Table 4 gives the concentrations of lead in in the ultrafiltrates, expressed in terms of % of the original plasma and serum concentrations. Each value represents the average of 2 determinations made on successive fractions of ultrafiltrate. The data indicate that citrate increases the percentage of diffusible lead, as thus determined, in plasma and in serum.

Discussion. These actions of citrate appear to be possible factors in the increased mobilization and excretion of lead observed in sodium citrate therapy. The fact that citrate promotes the removal of lead from plasma and from Ringer's solution, suggests that it may aid in the mobilization of the element by increasing the capacity of the cells to combine with lead

from the tissue fluid. This factor, coupled with the increase in the proportion of the diffusible form produced by citrate, should result in a freer exchange of lead between the tissues and the blood. The increased diffusibility should also make more of the element available for excretion.

factors. Citrate ion is known to exhibit a moderate indifference to cells. It is possible that its properties are so modified by the lead as to permit the complex to combine readily with the cells. However, it seems more likely that citrate ion serves as a carrier which takes up and then

TABLE 3.—EFFECT OF CITRATE ON THE ABILITY OF CELLS TO REMOVE LEAD FROM RINGER'S SOLUTION CONTAINING PHOSPHATE

Sample	Time before centrifuging (min.)	Citrate (mg./100 cc.)	Lead in cells (mg./100 cc.)	Lead in "plasma" (mg./100 cc.)	% lead in "plasma"
1	5	0	0.09	0.110	55
2	5	3	0.18	0.020	10
3	5	5	0.18	0.023	12
4	30	0	0.11	0.090	45
5	30	3	0.18	0.021	11
6	30	5	0.17	0.031	16
7	30	15	0.16	0.041	21
8	30	60	0.10	0.090	47

TABLE 4.—EFFECT OF CITRATE ON THE % OF DIFFUSIBLE LEAD IN PLASMA AND SERUM AS DETERMINED BY ULTRAFILTRATION

Citrate (mg./100 cc.)	Lead in plasma ultrafiltrate (%)	Lead in serum ultrafiltrate (%)
0	6	9
8	18	15
20	30	38

In considering these results in relation to therapy, it should be noted that the range of citrate concentrations employed in most of the experiments included some values which undoubtedly are considerably above those attained in the body. The administration of sodium citrate is known, however, to result in an elevation of blood citrate, and could be expected to influence the behavior of lead in the blood to a corresponding extent.

The experimental results are probably best explained on the basis of an increased reaction of citrate ion with lead ion to form the soluble complex,[7] in competition with other plasma constituents which also combine with lead. Additional evidence for the presence of this complex in solutions containing lead and citrate ions has recently been obtained by Bobtelsky and Jordan.[5] Although this explanation appears adequate to account for the observed reduction in the non-diffusible form in the plasma, the increased ability of cells to combine with lead may also involve other

releases lead ion to the cells. The latter view appears to find support in the observation that in Ringer's solution a relatively low concentration of citrate greatly increases the amount of lead taken up by the cells, while higher concentrations produce a smaller effect.

Summary. 1. The effect of sodium citrate on the distribution of lead between the plasma and cells, and on the proportion of diffusible lead in plasma and serum, has been investigated, using the isotope, radium D, as a radioactive indicator.

2. When lead is added to blood *in vitro* at such concentrations that a large fraction ordinarily remains in the plasma, the presence of added citrate increases the percentage of lead taken up by the cells.

3. The addition of citrate greatly increases the ability of the cells to combine with lead previously mixed with plasma or with Ringer's solution containing phosphate.

4. Lead is readily transferred from cells to plasma, but citrate has little or no effect on the process.

5. Added citrate reduces the proportion of non-diffusible lead in plasma and serum, as determined by ultrafiltration.

6. The possible bearing of these results on citrate therapy is briefly discussed.

I am indebted to Miss Margaret Anderson for technical assistance.

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ALTERATION OF THE ELECTROCARDIOGRAPHIC P WAVES IN ACUTE RHEUMATIC FEVER

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Although many reports have been made of electrocardiographic changes in patients with acute rheumatic fever,[8,10,12,13] there have been few detailed analyses of the frequency of various abnormalities or their changes during various stages of clinical activity of the disease. Wendkos and Noll[12] present a recent summary of most of the changes that occur during active rheumatic fever, including delay or deformity of the QRS complex, modification of the S–T segment and the T waves, as well as auriculoventricular conduction delay. During the 1943 epidemic of acute rheumatic fever at the United States Army Air Forces Station Hospital at Lowry Field, Colorado, these and certain other changes considered significant were observed. Since it has not received special comment previously, the modification of P waves observed in rheumatic fever is considered worthy of report.

It would be expected from the frequent extensive involvement of the atrial muscle that the electrocardiographic P waves would be modified during the active phases of acute rheumatic fever. It is believed that such changes commonly occur but that there has been hesitancy in either recognizing or reporting them because of the normal variations in form and direction of P waves, especially in Lead III. The respiratory cycle variations of P_3 is notably great.

Alterations of form, amplitude, duration and direction have not only been described in arrhythmias involving aberrations of atrial pacemaking and conduction, but likewise in mitral stenosis,[2,11] and pulmonary valve stenosis.[1] There have also been studies of the P waves in esophageal leads,[3,9] myocardial infarction,[7,9] and myocardial failure.[5] Recently there has been a detailed study of the electrical axis of P waves in normal subjects and in those with atrial arrhythmias.[4a,b,c] The variations in electrical axis seem to have been influenced more by the size and position of the auricles than by intrinsic changes in the myocardium affecting conduction.

P wave changes as herein reported in acute rheumatic fever cases are thought to be significant, especially when successive records show changes in form that seem to coincide with increased activity of the disease. Flattened, diphasic or inverted P_3 waves were not considered significant unless the abnormality was great enough to modify P_2 as well as P_3 and exhibit changing form on successive records. However, changes of lesser degree may be considered significant in future studies. The respiratory variation of P_3 was adequately discounted in evaluating the changes interpreted as abnormal. P_1 variations generally were accompanied by similar modifications of P_4 and of P_2 to an appreciable degree less frequently.

In 60 successive active acute rheumatic fever cases studied, out of 551 observed during the period of January 1943 through September 1943, 8 (approximately 11.7%) showed significant flattening or inversion of P waves. Five of these cases showed variations in successive records taken at weekly intervals and the flattening or inversion of these waves generally seemed to coincide with a period of activity, corroborated by clinical and laboratory evidences. Of the 8 cases, 5 presented flattening or inversion of P_2 and P_3, 2 demonstrated alterations in P waves in all leads including Lead IV F, and 1 showed only

P_1 and P_4 inversion. It is apparent from these cases that changes are more common in Lead III than in Leads I or IV F. In view of the greater prominence of auricular flutter waves in Lead III than in Lead I, probably due to the more perpendicular position of this lead to the average axis of the auricular vectors, it might be expected to reflect more sensitively the changes in auricular conduction. Auricular changes would likewise be expected in esophageal leads and such was demonstrated in 2 of the cases with abnormal P waves at 35 cm. and 40 cm. levels with loss of the initial upright phase of the sharp diphasic complex.

These alterations in P wave directions are apparently a sensitive index of cardiac involvement in that in only 2 out of the 8 cases were other unequivocal electrocardiographic abnormalities noted, namely a flattened T_1 in 1 case and prolongation of the auriculoventricular conduction period in another. Diphasic T_3, flattening of T IV F, low voltage of the QRS and right axis deviation occurred in 4 other cases but these changes could not be interpreted as definitely abnormal. Comment may be made that tendency toward right axis deviation was observed with unusual frequency in acute rheumatic fever patients, without evident valvulitis. No explanation is offered for this finding.

In addition to these 8 cases there were 7 others in the 60 studies in which minor P wave changes were observed but were not considered significant. Greater amplification of the waves may permit the development of more exact diagnostic criteria for such records, which now are not considered diagnostic.

Certain case histories serve to illustrate the relation of atrial changes in the electrocardiogram to the clinical activity of the acute rheumatic fever.

Case Histories. Case 1. Patient X, age 32, was admitted to the Station Hospital, Lowry Field on Aug. 4, 1943, with pain, swelling, redness and tenderness of both ankles and the right knee of 3 days duration. He had noted fatigue, dyspnea on exertion, and anorexia for the past 2 months, succeeding an upper respiratory tract infection. There was a past history of recurrent pains in both ankles and lower legs, between the ages of 6 and 9 years. On admission the oral temperature was 99.4° F., the pulse rate 100 per minute, the white blood count 8300 and the erythrocyte sedimentation rate 5 mm. per hour (Cutler technique). Roentgen films of the heart and lungs were normal. The electrocardiogram on Aug. 6, 1943, showed a low voltage in all leads, and a sharply inverted P_3 and slightly inverted P_4.

On August 19, at which time all joint signs had subsided and the temperature had returned to normal, the P_3 deflection had reverted to upright. The electrocardiogram on August 28 was unchanged except that voltage in all leads had increased slightly. The sedimentation rates repeatedly remained normal as did the leukocyte count. This case illustrates definite alteration of the direction of P_3 during clinical activity of acute rheumatic fever accompanied by low grade fever but no other laboratory evidence of activity of the disease.

Case 2. (Figs. 1 and 2.) Patient Y, age 19, was hospitalized at Buckley Field Station Hospital from July 2 to July 28, 1943, with a characteristic initial attack of acute rheumatic fever. He was admitted to Lowry Field Station Hospital on July 29, 1943, with swelling, redness and tenderness of the knees, ankles and right shoulder and moderate precordial pain, increased by cough. No other abnormal physical findings were present. The temperature was 99° F. and the pulse rate was 96 per minute. The erythrocyte sedimentation rate was 1 mm. in 60 minutes. The Roentgen ray film of the chest showed normal lung fields and prominence of the left cardiac border. The electrocardiogram on July 30 showed inversion of P_2 and P_3.

The course continued unchanged for 2 weeks with an occasional elevation of temperature from 99.2° F. to a maximum of 100.2° F., slight tachycardia and joint pains, but repeatedly normal leukocyte counts and erythrocyte sedimentation rates.

The temperature remained normal from mid-August through the 1st week of September, there was improvement in the signs of joint involvement and the electrocardiogram showed upright P_2 and P_3 on August 30. A slight increase of joint activity and mild fever occurred in early September and on September 14, P_2 again became inverted. The electrocardiogram on October 9 showed

some inversion of P_2 and sharp inversion of P_3 as on admission to the hospital, although no definite clinical change was noted. On October 23, although a progressive improvement was noted clinically, the inversion of P_2 and flattening of P_2 persisted.

These waves became upright on October 27 but again showed deep inversion on October 30. In early November clinical improvement was appreciable and the patient was first allowed to sit up on November 15. The electrocardiogram of November 18 showed a further return to upright of P_2 and P_3. Successive records showed no alteration during convalescence.

This case illustrates fluctuating changes in the P waves in Leads II and III, at times varying with the obvious clinical course, and at others alternating direction rather abruptly during a recovery phase, without accompanying signs of increased joint activity. In this patient there was likewise poor confirmation of the obviously active arthritis by consistent fever, increased leukocyte count or prolonged erythrocyte sedimentation rate. No significant electrocardiographic changes occurred other than in the P waves.

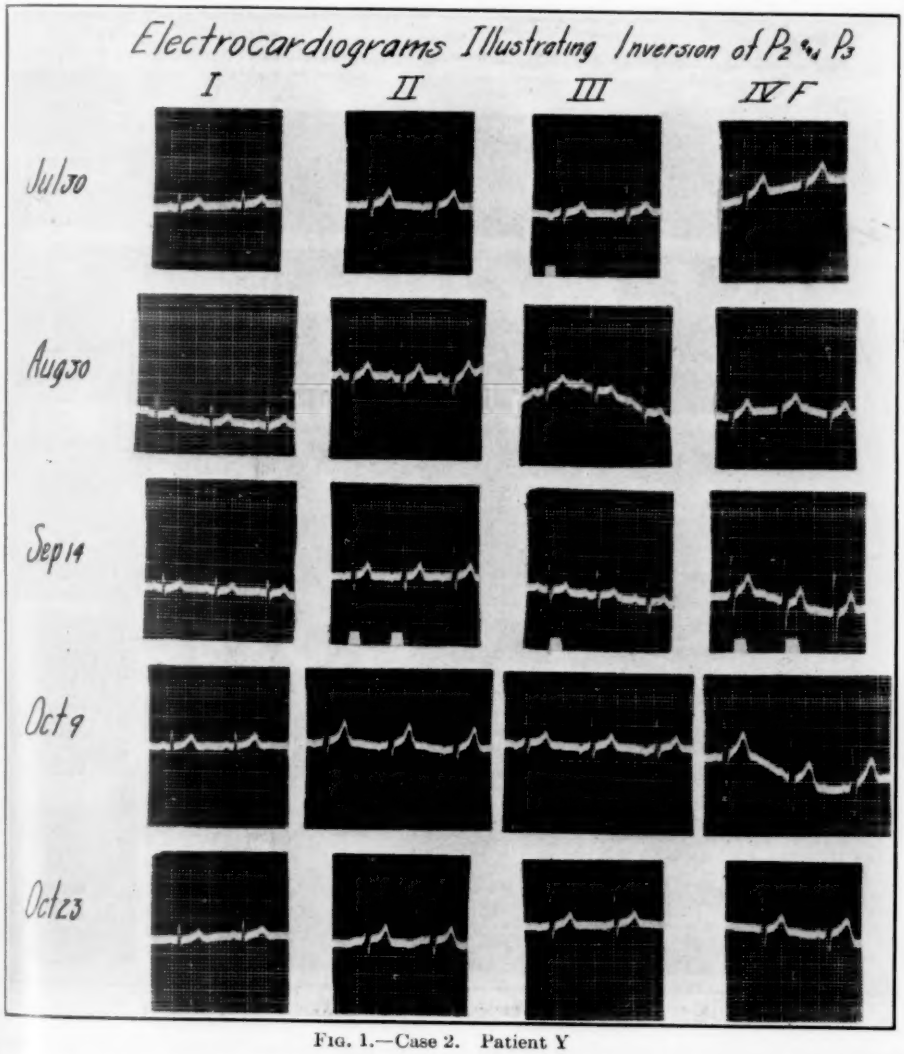

FIG. 1.—Case 2. Patient Y

CASE 3. (Fig. 3.) Patient Z, age 20, was admitted to Lowry Field Station Hospital on July 23, 1943, having had a moderately

severe sore throat 3 weeks previously. Two days prior to admission he developed painful, reddened, swollen ankles. On admission his temperature was 99.2° F., pulse rate 102 per minute, leukocyte count 9400 and an erythrocytic sedimentation rate of 25 mm. per hour (Cutler technique). The electrocardiogram on July 28 showed inversion of P_1 and P_{4F}. Salicylate therapy was instituted and the joint involvement slowly subsided within the next month but the erythrocytic sedimentation rate was still 28 mm. per hour on August 16. The electrocardiogram was unchanged on August 26, with persistent inversion of P_1 and P_{4F}. The erythrocytic sedimentation rate fell to 14 mm. per hour on September 22 and to 4 mm. per hour on October 6. The electrocardiogram on September 2 and 21 showed upright P_1 and flattened P_{4F}.

There was obvious chronic tonsillar infection and 2 abscessed teeth. The latter were removed on October 9 and the former on October 28. He was transferred to the convalescent ward on November 3, where he remained free of joint symptoms until November 13. On that day he developed pain and swelling of the right knee and on November 18, of the right shoulder. His temperature varied from 100.2° to 101° F.,

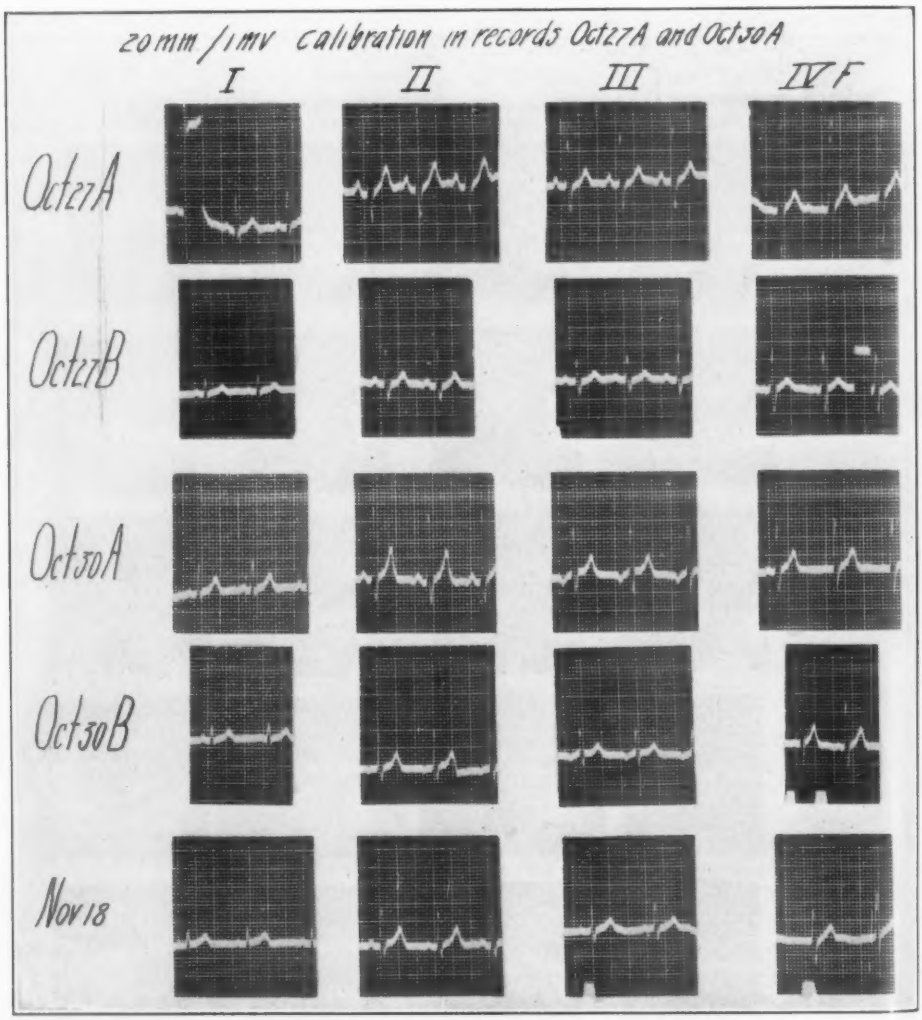

FIG. 2.—Case 2. Patient Y.

and pulse rate from 96 to 104 per minute in those 5 days but both returned to normal by November 22 under resumption of salicylate therapy.

On November 17, his erythrocyte sedimentation rate was again 27 mm. per hour and his leukocyte count was 15,200. The electrocardiogram demonstrated inversion of T_1 and T_4. He gradually improved in the succeeding month, and on November 22 his electrocardiogram showed isoelectric P_{4F} and upright P_1, the white blood count being 7600 on November 23. On December 16, the standard electrocardiograph leads were unchanged but the esophageal leads, taken by Lt. Col. Leonard Williams, showed a monophasic inverted P wave at the 35 cm. and 40 cm. levels.

This case illustrated changes in P_1 and P_{4F} occurring concomitantly with an initial acute rheumatic arthritis, subsiding and reoccurring with a relapse following tonsillectomy and extraction of teeth. The P wave changes receded to normal in this patient prior to the fall of the erythrocyte sedimentation rate to normal levels. No other significant deviations from the normal electrocardiogram were observed in this case.

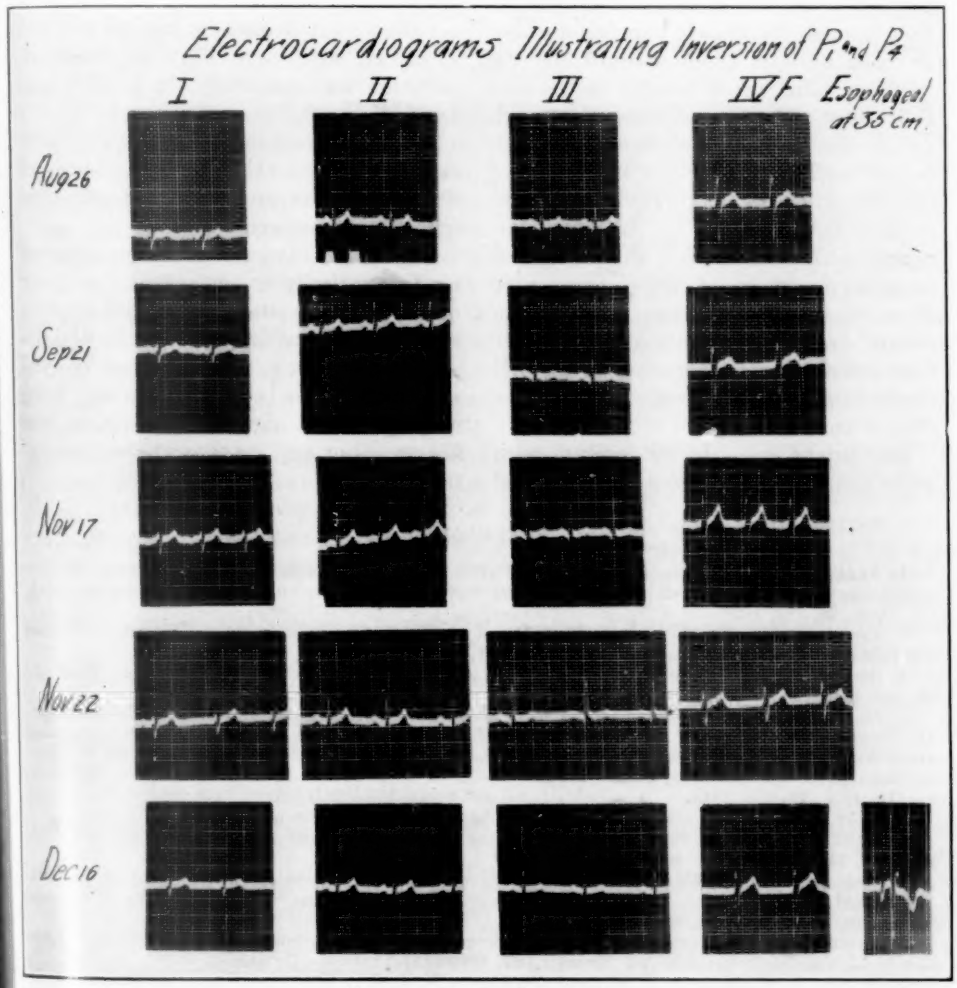

FIG. 3.—Case 3. Patient Z.

No conclusive explanation can be offered for the development of these changes in the atrial electrocardiographic waves.

There have been minor differences of P–R conduction time observed in some of the records, but these differences were not sufficiently consistent to indicate a positive relation to the causal mechanism of the deviations of the P waves. Modification of the quality or direction of conduction through the muscle bundles of either or both auricles could account for the findings. This would notably be the case if it were generally accepted that such conduction takes place through certain bundle pathways and that inequality of contraction of the 2 auricles is not a rare phenomenon (Luisada[6]). It seems improbable that alteration of the pacemaker accounts for most of these progressive P wave changes,* although rarely an abrupt transient alteration of P wave form associated with variation in the P–R conduction time occurred, suggesting a shift of pacemaker.

Conclusions. 1. In 60 patients with acute rheumatic fever selected for detailed study at the AAF Station Hospital at Lowry Field, Colorado, significant changes in the P waves of the electrocardiogram were observed in 8 of the patients and questionable alterations in 7 others.

2. These changes consisted of distinct flattening or inversion of the P waves and occurred most frequently in Lead III and Lead II, but were likewise observed, although less commonly, in Lead I and Lead IV F.

3. The alterations in the P waves generally paralleled the clinical evidences of active joint involvement and subsided with clinical recovery.

4. These P wave deviations are regarded as diagnostically important in that they often occurred unaccompanied by other electrocardiographic abnormalities and occasionally during periods when clinical signs of active rheumatic fever were associated with normal temperature, leukocyte count and erythrocyte sedimentation rate.

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* Since submission of this article for publication a comprehensive review has been published by Filberbaum, M. B., Griffith, A. C., Solley, R. F., and Leake, W. H.: "Electrocrrdiographic Abnormalities in 6000 Cases of Rheumatic Fever;" Calif. and Western Med., **64**, 340, 1946. These authors found 1.63% of patients demonstrating P' wave changes generally in Leads I and II. They favor a shift of pacemaker, to account for these changes but do not correlate change in P–R interval with change in n form of P waves.

EVIDENCE OF MATERNAL RH SENSITIZATION WITHOUT EVIDENCE OF HEMOLYTIC DISEASE IN THE NEWBORN

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It has been repeatedly stated[2,3,4,8] that hemolytic disease of the newborn can occur without the presence of demonstrable Rh antibodies in the maternal serum. The converse of this statement has, however, appeared to our knowledge only twice in the literature up to the present time. Dockeray and Sachs[6] reported 4 cases in which they were able to demonstrate what appeared to be Rh iso-agglutinins in the Rh negative mother's sera, yet these same mothers delivered 4 apparently normal Rh positive infants. The second report of this nature was by Goldbloom and Lubinski,[7] who record 1 example of Rh iso-immunization in the mother without symptoms of hemolytic disease in the infant.

The purpose of this paper is to describe 2 additional cases in which we were able to demonstrate Rh iso-antibodies in the maternal sera of Rh negative mothers without finding evidence of hemolytic disease of the newborn in their Rh positive offspring, and to discuss 2 mechanisms by which such apparent paradoxes could occur.

Case Reports. Case 1. Mrs. T. is a 31 year old para I, gravida II, Wassermann reaction negative. Her first pregnancy terminated in a criminal abortion at about 2 months of pregnancy, 5 years ago. The second pregnancy was entirely uneventful and terminated by the mid-forceps delivery of a living 3980 gm. male infant on Oct. 9, 1944. The infant appeared to be perfectly normal at the time of birth. The placenta weighed 690 gm., and was normal in appearance. On October 18, we received a sample of the mother's blood for Rh determination. This blood was brought to the laboratory merely because it was hospital routine to do Rh determinations on all obstetrical patients, not because of any clinical indication for the determination. At this time we found the mother to be Group A Rh negative and to have Rh agglutination antibody in her serum (Table 1). A second sample of the patient's blood obtained 2 days later, for checking, confirmed the findings on the original sample. Titration of the antibody in the second sample showed an anti-Rh titer of 1:4096. Incidentally, this is the highest titer we have observed in doing more than 2000 determinations for anti-Rh agglutinins.

At the time the antibody was found in the mother's serum, the infant was 10 days old. No blood studies had been carried out on the baby, but there had been no indication for these. In checking back with the pediatrician in charge of the case, as well as the

Table 1.—Blood Groups and Antibodies of the T Family

	Date	Group	Rh	Maternal Rh antibody titer in saline*	Maternal Rh antibody titer in serum†
Mr. T.	3/10/45	A	Rh_1		
Baby T.	10/18/44	A	Rh_1		
Mrs. T.	10/18/44	A	Neg.	1:4096	
	3/10/45	..	..	1:32	
	9/24/45	..	..	1:32	
	1/19/46	..	..	1:32	1:256

* Maternal serum added to 2% suspensions of Rh positive test cells suspended in saline. Incubation. Read for agglutination.

† Maternal serum added to 2% suspensions of Rh positive test cells suspended in compatible serum. Incubation. Read for "conglutination."[7]

obstetrician and interne, nothing abnormal was noted concerning the baby at birth nor during the first 10 days of its life. The only positive finding was a note that the baby was slightly icteric on October 14, the 6th day of life. This icterus had faded by the next day, and was interpreted as physiologic jaundice. The baby was discharged home on its 12th day of life at which time examination was entirely normal. The weight of the infant at the time of discharge was 4112 gm., a gain above birth weight of 130 gm. The infant was seen at frequent intervals from that time until it was over 18 months of age, and seemed to be normal at all times.

Typing of the infant's blood revealed it to be Group A Rh positive, and of the father's blood revealed him to be Group A Rh positive.* The titer of Rh agglutinin in the mother's serum has fallen since delivery, as shown in Table 1.

Admittedly the infant might have had subclinical hemolytic disease of the newborn, if such an entity exists. But, if one believes that disease is a definite morbid process having a characteristic train of signs and symptoms, then this infant was normal. It exhibited no edema, pallor, hepatomegaly, splenomegaly, bleeding tendencies, abnormal weight loss, nor feeding difficulty, but rather appeared to be a perfectly healthy baby in every way. However, it must be admitted that no blood studies were done on the infant, and also that icterus was present, but not until the 6th day of life, and lasted only about 24 hours. Certainly this would not be universally accepted as a sign of hemolytic disease of the newborn. The placenta was normal, and the ratio of its weight to that of the baby was normal, *i. e.*, 1:6.

The reason for the extremely high agglutinin titer is not clear. The patient had had 1 previous pregnancy, but this had been criminally interrupted at 2 months and hence it is unlikely that the mother received any great amount of sensitization to the Rh factor through that pregnancy. She has never received blood intravenously nor intramuscularly. It is conceivable that the titer was not as high during the pregnancy as we found it to be 10 days postpartum. Unger and Wiener[9] believe that at the time of placental separation there is a sudden shower of fetal red cells into the maternal circulation and that this is a potent factor in sensitizing the Rh negative mother against the Rh factor. If this hypothesis is true, we have then at least a partial explanation for the high titer observed in this case.

It is of interest to note that this patient's serum contained agglutinins of both the variety detectable in a saline system as well as the variety demonstrable in a serum or albumin system.[5,10] The latter variety was not tested for until over 1 year postpartum, but its presence at that time is certainly indicative of its presence at the time of delivery. It is believed[5] that agglutinins of the variety demonstrable only in serum or albumin systems exert a much more serious effect on the infant than those of the simple variety. Yet this infant exhibited none of the characteristics of hemolytic disease of the newborn.

Case 2. Mrs. M.† is a 22 year old para II, gravida III, with expected date of confinement Jan. 25, 1946, Wassermann reaction negative. Her first 2 pregnancies ended by the delivery of full-term normal infants. The patient had never been transfused. We first came in contact with the case on Dec. 4, 1945. At that time we found the patient to belong to Group A and to be Rh negative (Table 2). In addition, Rh antibody of the type demonstrable in serum or albumin systems was found in her serum in titer of 1:16. None of the simple variety of antibody was demonstrable. The rise in the former type of antibody titer is recorded in Table 2 along with the subsequent appearance of the simple variety of Rh antibody and other pertinent findings.

On Jan. 28, 1946, the patient delivered a normal full-term infant weighing 3650 gm. The placenta was normal grossly and microscopically.

* The blood samples on all 3 members of this family were subsequently referred to Dr. Louis K. Diamond for checking and he reports as follows: Mr. T.: Group A Rh_1 homozygous. Baby T.: Group A Rh_1 heterozygous. Mrs. T.: Group A Rh negative. Specificity of Rh antibody from Mrs. T. is of Rh_0 variety.

We are indebted to Dr. Diamond for carrying out these tests.

† We are indebted to Dr. Floyd C. Bratt, Rochester, N. Y., for the opportunity of studying this patient and to the Genesee Hospital, Rochester, N. Y., for making available the hospital records of this case.

The baby was observed carefully during the first 8 days of life and at no time was there any evidence of a hemolytic process present. Blood studies are shown in Table 3. There was no edema, pallor nor icterus. The infant took its feedings well, and was discharged home on its 8th day of life, weighing 200 gm. below birth weight. Careful follow-up since that time has revealed normal progress.

Lubinski[7] state that the amount of antibody which passes through the placenta from the mother's circulation into the blood stream of the fetus varies widely in different cases, but they offer no explanation for this variation. They also believe that unknown factors in the fetus which they call "disposition" may be of importance.

TABLE 2.—BLOOD GROUPS AND ANTIBODIES OF MR. AND MRS. M.

	Date	Group	Rh	Maternal Rh antibody titer in saline*	Maternal Rh antibody titer in serum†
Mr. M.	1/10/46	O	Pos.		
Mrs. M.	12/ 4/45	A	Neg.	..	1:16
	1/10/46	..	..	..	1:16
	1/22/46	..	..	1:1	1:32
	1/28/46	..	..	1:4	1:32
	3/13/46	..	..	..	1:16

Pregnancies				
No.	Year			
1	1943	3/13/46	O	Pos.
2	1945	3/13/46	O	Neg.
3	1946	1/28/46	O	Pos.

* Maternal serum added to 2% suspensions of Rh positive test cells suspended in saline. Incubation. Read for agglutination.

† Maternal serum added to 2% suspensions of Rh positive test cells suspended in compatible serum. Incubation. Read for "conglutination."[7]

TABLE 3.—BLOOD STUDIES ON BABY M (CASE 2)

Date	RBC (mill. per c.mm.)	Hb. (gm. per 100 cc.)	Smear (normoblasts per 100 WBC)
1/28/46	6.28	17.6	2
1/29/46	8.12*	25.8	1
2/ 1/46	6.47	20.6	
2/ 5/46	6.10	16.7	

* High levels probably due to dehydration.

It should be noted that the maternal serum contained Rh agglutinin of the type demonstrable only in serum or albumin systems almost exclusively and it was not until about 1 week before delivery that any of the simple variety was found.

Discussion. The birth of normal Rh positive infants from Rh negative mothers who have been sensitized to the Rh factor in the red cells of their fetuses can probably not be explained by our present knowledge of the forces involved. Dockeray and Sachs[6] state that perhaps the Rh antibodies pass the placental barrier only in certain conditions changing during the course of pregnancy. Goldbloom and

There occurs to us 2 other possibilities: 1. Witebsky[12] has studied the occurrence of Rh substances in amniotic fluid and concludes that certain individuals are secretors of Rh and certain others are non-secretors. He bases this conclusion on the presence or absence of the Rh substances in amniotic fluids. He reports 3 cases of hemolytic disease of the newborn in which the mothers were Rh negative, the infants Rh positive and were non-secretors of the Rh factor. Boorman and Dodd[1] have found the Rh substance in tissue cells as distinct from the red blood cell.

Maternal and fetal incompatibility of the major blood groups is of very frequent

occurrence, yet hemolytic disease as a result of this incompatibility is quite infrequent. The most logical explanation for this infrequency is that the vast majority of individuals (±80%) are secretors of the A or B factors and hence the antibody which the fetus obtains from its mother is to a large degree neutralized by the bulk of the body tissue, and only a relatively small amount is left free to react on the fetal red cells.

If this same logic is applied to the cases under discussion, we might say that these babies were secretors of the Rh factor, and hence the Rh antibodies which reached them by way of the placenta were largely taken up by the bulk of fetal tissue, and such a small amount remained to react with the red blood cells, that they were not sufficiently damaged to result in hemolytic disease. Unfortunately, no attempt could be made to study the amniotic fluid in either of the cases presented.

2. Wiener[11] in describing his "conglutination" test states that it occurs in 2 stages, and that a third component besides the Rh antigen and the specific antibody is necessary for the reaction of clumping to occur. He describes this third component as a colloidal substance in the plasma, possibly identical with the so-called X-protein, and calls it "conglutinin." He believes that in those infants which do not present signs of hemolysis for several hours or even days after birth, the "conglutinin" does not form until after birth, and that when it finally does form, the infant then shows evidence of hemolytic disease.

By similar reasoning one might say that in certain cases when normal Rh positive infants are born to Rh negative mothers whose serum contains Rh antibodies, those infants are normal because the particular antibody in the maternal serum cannot react with the antigen on the fetal red cells because of the absence of the third necessary component, "conglutinin." This could possibly be the explanation in the second case presented since only the "conglutinating" type of antibody was present in the maternal serum until the last week before delivery at which time a small amount of the simple variety of antibody was also found.

We believe that cases of the type described here and previously are of interest only to workers in the field. Obstetricians and pediatricians should continue in the belief that when Rh antibodies are found in the serum of a pregnant woman, that woman will probably deliver an infant with hemolytic disease. Believing this, he should do everything possible to lessen the severity of the disease in the newborn, *i. e.*, induction of labor when indicated, followed after birth by immediate and repeated transfusions of Rh negative blood to the infant, or by very close observation, including red blood cell counts and hemoglobin determinations twice daily for the first 5 or 6 days of life, then daily for the next week. These babies, of course, should not receive the mother's milk.

Summary. 1. Two cases are presented in which apparently normal Rh positive infants were born to Rh negative mothers whose sera contained Rh antibodies.

2. Two possibilities to explain these unusual cases are discussed.

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RESPONSE TO PENICILLIN BY A TYPHOID CARRIER

STUDIES ON PENICILLIN SENSITIVITY OF STRAINS OF TYPHOID BACILLI

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PENICILLIN now has become more readily available and recently it has been possible to treat 2 typhoid carriers with fairly large doses of the substance. It is believed that 1 of the patients, found to be a very definite gall bladder carrier, was apparently cured as the result of treatment while the other, whose focus of infection was not as definite, received only a temporary effect. In an effort to explain this apparent cure with penicillin, a collection of various strains of *Eberthella typhosa*, including that of the successfully treated carrier, was analyzed for *in vitro* susceptibility to penicillin. It was possible to demonstrate some differences between the organism of the patient and those from other sources. Also, it is thought that the ability of the liver to concentrate penicillin was responsible in part for the good result achieved in the patient.

The history and treatment of the patient displaying a good response is as follows:

Case Report. CASE 1. A. C., a Negro male, age 31, was discovered to be a typhoid carrier following a routine check by rectal swab of food handlers on a Navy base. On investigation, it was found that he had been apparently involved in an outbreak of typhoid fever in his home district of Louisiana in 1927 when he and members of his family were ill at the same time with a disease known as "typhoid malaria." He had an uneventful recovery, and believed himself entirely well thereafter. He held various jobs as a laborer and enlisted in the Navy as mess steward in January of 1944. When discovered to be harboring *E. typhosa* he was working as a food handler in an officer's mess, this being his first work in handling food. No typhoid fever had been found in any Naval personnel of the base and the man had not been on leave. Rectal swab cultures on bismuth sulfite or S. S. agar taken at anytime showed almost pure cultures of typhoid bacilli, and bile cultures were also markedly positive, displaying pure growths of typhoid on each culture. Urine cultures were negative.

A Graham test demonstrated reduced function of the gall bladder, with at least 1 small calcified stone present but the patient reported no symptoms in the past suggestive of gall bladder disease. Because of the markedly positive bile cultures it was decided that his major focus of infection was in the gall bladder and operation was proposed. This was firmly refused by the patient.

During the 4 month period prior to the institution of penicillin therapy, various recommended therapies had been attempted without success. A course of sulfadiazine, consisting of 32 gm. in a 5 day period, was given the patient, but there was no apparent effect, rectal cultures remaining positive during and after treatment. A 5 day course of gall bladder dye was then administered, but this also showed no effect on his positive cultures.

When it was found by *in vitro* studies of the patient's organism that complete inhibition occurred on blood agar plates with penicillin levels of 8 to 9 Oxford units per cc. of blood agar, penicillin therapy was administered according to the following dosage:

While receiving penicillin, fluids were

*Other members assisting were: Lt. (jg) F. S. Greenspan, (MC) USNR; Lt. (jg) F. P. Ermilio, (MC) USNR; Lt. (jg) F. G. Schiro, (MC) USNR; Lt. D. L. Truax, H(S) USNR; Lt. (jg) T. D. Brown, H(S) USNR; Lt. (jg) M. Hessney, H(W) USNR; Ens. A. J. Johnson, H(S) USNR; PhM 1/c D. A. Treat; PhM 2/c F. W. Elias; PhM 3/c D. Morrison.

limited to 800 cc. daily and the patient was allowed to have the regular hospital diet.

After 2,350,000 units of penicillin had been administered over a 7 day period the patient refused to go on with treatment. He was returned to duty and observed but was not allowed to handle food. Since the termination of penicillin treatment the patient has been asymptomatic and has remained free of typhoid organisms. He has now shown negative rectal cultures for a period of 5 months. Cultures of bile were negative for typhoid 2 and 5 weeks after the end of treatment. Rectal swab cultures always had been markedly positive for *E. typhosa* during the 4 month period while he was under observation prior to receiving penicillin.

Rectal swab examinations were consistently positive for typhoid. A bile examination revealed only 4 to 5 colonies of typhoid per cc. of bile and there was some doubt as to whether or not he was a gall bladder carrier. Penicillin sensitivity tests were carried out with this strain as recorded in Tables 3 and 4, and he was later placed on the following régime of penicillin therapy as a trial.

Unfortunately, orders to limit the patient's fluids to 800 cc. daily were not carried out and the patient drank a normal intake each day.

After receiving 3,450,000 units of penicillin over a 9 day period this patient showed negative cultures temporarily (*i. e.*, for a period of 1 week) but cultures later became positive and have remained so. Since it was necessary to transfer the patient to another hospital, he was no longer available for a retrial with penicillin. Further attempts at therapy using a longer period of administration of larger doses of penicillin were felt to be probably justified, because of the temporary negative cultures found.

TABLE 1.—INHIBITION OF GROWTH BY PENICILLIN (CASE 1)

Day	Date	Total daily dose (Oxford units*)	Cultures		
			Rectal swab	Bile	Urine
	12/ 7/44–4/11/45	...	8 Pos.	2 Pos.	1 Neg.
1	4/11/45	200,000			
2	4/12/45	400,000			
3	4/13/45	400,000	Less than 5 colonies per plate		
4	4/14/45	400,000	"		
5	4/15/45	400,000	"		
6	4/16/45	400,000	Neg.		
7	4/17/45	150,000	"		
	Total:	2,350,000			
13	4/23/45	...	"		
17	4/27/45	...	"		
24	5/ 4/45	...	..	Neg.	Neg.
35	5/15/45	...	Neg.		
49	5/29/45	...	"	Neg.	
55	6/ 4/45	...	"	..	Neg.
101	7/20/45	...	"		
137	8/25/45	...	"		

* 50,000 units intramuscularly every 3 hours.

CASE 2. This case reported having had typhoid fever 3 years ago. This man, H. B., a 25 year old German prisoner of war was first discovered to be a typhoid carrier during an outbreak of typhoid fever in a prisoner of war camp. After an investigation had been made, it was concluded that he was responsible for 3 cases of typhoid fever in his fellow prisoners, with 1 of the cases ending fatally. The original infection of the carrier was contracted while he was fighting on the German Front in Russia. Since then he had believed himself well and, before the present outbreak, had noted no cases of typhoid in his vicinity.

The possibility was considered that strain variation in susceptibility of *E. typhosa* might exist and that, if such were found, a possible explanation would be available for the apparent cure of 1 of the carriers. *In vitro* studies were made on a

group of various strains of typhoid organisms.

Eight different strains of *E. typhosa* were obtained and simultaneous evaluation made for the effect of varying concentrations of penicillin upon 24 hour broth cultures of the different organisms. None of the patients furnishing the strains had ever received penicillin.

TABLE 2.—INHIBITION OF BACTERIAL GROWTH BY PENICILLIN (CASE 2)

Day	Date	Total daily dose (Oxford units*)	Cultures		
			Rectal swab	Bile	Urine
0	8/14/45	...	2 Pos.	Pos.	Pos.
1	8/24/45	250,000			
2	8/25/45	400,000	Pos.		
3	8/26/45	400,000	"		
4	8/27/45	400,000	"		
5	8/28/45	400,000	..	Pos.	Neg
6	8/29/45	400,000	Pos.		
7	8/30/45	400,000	Neg.		
8	8/31/45	400,000	"		
9	9/ 1/45	400,000			
	Total:	3,450,000			
10	9/ 2/45	...	"		
13	9/ 5/45	...	"		
20	9/12/45	...	Pos.		
21	9/13/45	...	"		
22	9/14/45	...	"		

* 50,000 units intramuscularly every 3 hours.

TABLE 3.—DEGREE OF GROWTH* OF TYPHOID BACILLI IN BROTH CONTAINING VARYING AMOUNTS OF PENICILLIN

Oxford units of penicillin per cc. of broth	A. C.†	H. B.‡	A. G.	T. B.	C.	D.	E.	F.
0	4	4	4	4	4	4	4	4
5	4	4	4	4	3	4	3	4
10	3	3	3	3	2	4	2	4
15	2	3	3	3	2	4	2	4
25	2	1	2	1	1	3	1	2
30	2	1	2	2	1	3	1	1
50	1	1	1	1	1	2	1	1

* Degree of growth in broth graded 1 to 4.
† Organisms from carrier successfully treated.
‡ Organisms from carrier showing only temporary response.

(Origin of typhoid strains: A. C.: phagetype "A" strain from typhoid carrier who, under treatment, made a favorable response to penicillin; H. B.: strain from German P. O. W. carrier unsuccessfully treated with penicillin; T. B.: strain from mild case of typhoid fever in German P. O. W. outbreak; A. G.: strain from German P. O. W. dying of typhoid fever; C: phagetype "C" from carrier; D: organism from a urinary carrier; E: laboratory strain; F: laboratory strain.)

There is a definite difference noted in strain susceptibility to penicillin, especially when the higher concentrations of penicillin are added to broth cultures, but it is presently unknown whether or not these inhibitory concentrations of penicillin ever could be attained in a human gall bladder carrying typhoid organisms. It is of interest, however, that while one gall bladder carrier, A. C., received apparent beneficial effect from penicillin, his organism by this type of *in vitro* examination displayed no startling difference from that of the resistant carrier, H. B.

The incubation of tubes of broth and penicillin that have been inoculated with typhoid organisms clearly does not repre-

sent *in vivo* methods; much, therefore, remains conjecture. A different method of demonstrating strain variation consisted of inoculating a loopful of a 24 hour broth culture of a particular strain upon the surface of a blood agar* plate containing various amounts of penicillin.

Table 4.—Growth* of Strains of Typhoid Bacilli on Blood Agar Plates Containing Varying Amounts of Penicillin

Oxford units per cc. of blood agar	A. C.†	H. B.‡	A. G.	T. B.	D.	E.	F.
4.1	2	1	3	..	4	1	
5.0	2	3	4	1	4	1	3
5.3	1	4	1	0	4	0	
6.8	1	2	1	2	4	0	2
7.6	1	1	1	1	1	0	1
8.4	1	0	1	1	1	0	
9.0	0	0	0	0	0	0	
9.8	0	0	0	0	0	0	

* Degree of growth graded from 1 to 4.
† Organism from carrier successfully treated.
‡ Organism from carrier showing only temporary response.

In this series it is of interest to note that much smaller amounts of penicillin, when in a blood plate, cause total inhibition of growth. However, the same relative difference in strain susceptibility was again noted. The "D" strain proved the most resistant and the "E" strain the most sensitive. The "A. C." strain, from the carrier successfully treated with penicillin in both series, demonstrated a sensitivity in the middle ranges, but when on a blood plate it appeared somewhat more sensitive than the organism of the carrier H. B., the latter displaying only a temporary response to treatment.

Comment. It has been stated[2,3,5] that penicillin is ineffective in the treatment of typhoid fever and other infections with gram negative bacilli, but in the asymptomatic and persistent carriers of typhoid organisms, and especially in those individuals who possess a focus of typhoid infection in the gall bladder, factors may be present which allow a response to the effect of penicillin.

Abraham and Florey[1] in early work with penicillin reported an interesting effect on typhoid and other organisms. They found that, though a concentration of penicillin far above that obtained therapeutically might be needed for microscopic inhibition of broth cultures of typhoid, smaller concentrations of penicillin would still cause microscopic changes in the appearance of the organisms, even though the organism survived. Exposure of broth cultures to dilutions of penicillin containing a concentration one-sixth that of the total inhibiting dose would bring about definite microscopic changes in structure and it was intimated that possible therapeutic effects might be obtained by these same high dilutions of penicillin. Hobby[4] was able to demonstrate a relative sensitivity of typhoid organisms to penicillin and Thomas[8] observed unusual structural forms of gram negative bacilli after exposure to dilutions of penicillin greater than those needed for complete bacteriostasis. Steiner[7] also described considerable variation in sensitivity to penicillin of various gram negative bacilli.

The findings by Abraham and Florey,[1] that higher concentrations of penicillin occurred in cat's bile than in the blood serum of the animal, suggested that a beneficial effect might be obtained in treating gall bladder typhoid carriers with penicillin. Rammelkamp[6] was able to show that penicillin was present in human bile in higher concentrations than in blood serum and concluded that penicillin is perhaps concentrated by the liver. Such a concentrating power might possibly allow effective penicillin levels to be

* Nutrient agar plus 10% by volume of defibrinated sheep's blood made up as a pour plate. Penicillin added prior to pouring plate.

reached in treating gall bladder typhoid carriers.

Considerable work has appeared demonstrating variations in susceptibility to penicillin of strains of staphylococci and other organisms. The susceptibility of certain strains of the typhoid bacillus as demonstrated above could be a third factor for the successful treatment of a typhoid carrier, especially one whose infection is primarily in the gall bladder. However, it is presently unknown whether or not the inhibitory concentrations of penicillin required for susceptible strains as demonstrated by *in vitro* methods could ever be attained in a human gall bladder.

Still another factor that might be responsible for a penicillin effect on typhoid organisms would be the presence in certain lots of ordinary commercial penicillin of unusual amounts of substances of the streptomycin type, or related groups. The penicillin used in treating the carrier "A. C." was manufactured by the Cutter Company and was produced by the shallow flask method.

It is admittedly very difficult to determine when a typhoid carrier is cured, but in the patient A. C. mentioned above, there has been an apparent response to penicillin therapy which has persisted for 5 months. Carriers have relapsed after such an interval but the response made, even if in only 1 patient, justifies further trials with similar therapies on larger groups of patients.

Summary and Conclusions. 1. A typhoid carrier of the gall bladder type made a response to large doses of penicillin and has remained free of typhoid bacilli for 5 months.

2. A second typhoid carrier similarly treated with penicillin made only a temporary response.

3. Among the strains of typhoid organisms tested, strain variation in susceptibility to penicillin was found to occur and this variation was not the result of previous treatment with penicillin.

4. Large doses of penicillin may be considered to be of value in the treatment of the typhoid carrier, but possibly only in the gall bladder type of carrier who has an organism sensitive to penicillin.

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BACTERIOLOGIC, CLINICAL AND PATHOLOGIC EXPERIENCE WITH 86 SPORADIC CASES OF SALMONELLA INFECTION*

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At the Queens General Hospital, 86 cases of Salmonella infection have been found since 1941, from which 21 different species of Salmonella have been isolated. The distribution, the type of syndrome, the severity and the sources of the various species are indicated in Table 1. The species with the highest incidence, *S. typhi murium*, *S. choleræ suis*, and *S. oranienberg*, accounting for 11, 20 and 16 cases respectively, showed interesting differences as to source, severity of symptoms, the absence of symptoms, or the development of the carrier state. There were 13 deaths, with 9 autopsies. Of the 86 cases, 19 had gastro-intestinal symptoms, 9 had general symptoms only, and 27 had both gastro-intestinal and general toxic manifestations. In addition to the 13 patients who died, 22 patients were severely ill and almost an equal number, (21) had only mild symp- The microörganisms were recovered from toms. the stool in 70 instances, from the blood in 11, and from the urine in 3, the others representing double infections.

Method and Material. The method for isolating enteric pathogens has been in use in this laboratory since 1941, and has been described by Mollov *et al.*[5] Inoculations were made simultaneously on one plate of S.S. agar, on plated MacConky media and in one tube of tetrathionate broth. Growth from this last was subcultured on MacConky agar only. From each plate, 2 to 4 of each type of non-lactose fermenting colonies were subcultured to Krumwiede triple sugar slants. One positive or suspicious slant from each case was studied for detailed fermentation reactions. All Shigella species were identified serologically and checked by the New York City Department of Health. After biochemical fermentation determinations, the Salmonella were identified serologically by the Beth Israel Hospital Salmonella Typing Center.

A total of 3206 stools were examined; 752 from hospitalized patients with intestinal disorders, and 2454 specimens from dietary workers, nurses and interns, all apparently in good health.

During this 4 year period, typhoid bacilli were isolated 19 times; 16 from patients ill with the disease and 3 from carriers (2 convalescent—1 for 30 years; and 1 a healthy carrier).

In most of the individuals from whom a species of Salmonella was isolated, more than 1 positive stool was obtained, the average being 3 positives. Of the 86 individuals harboring Salmonella microörganisms, 55 were hospital patients (27 females and 29 males), and 30 were from the surveyed dietary workers, nurses and interns. Of these, only 2 were males while 28 were females, because of the predominance of female nurses in this latter group.

Stools were studied routinely while blood and urine cultures were made only when particular indications existed.

Cultural and Clinical Findings. A wide range of Salmonella species was implicated. In general, the gastro-intestinal syndrome was most frequent, and the microörganisms were found most often in the feces. The variations in the syndromes and in their severity were remarkable when the infections were due to different species, but were also quite marked when different cases of infection with the same species were compared. The clinical variations were also found in instances when several

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simultaneous infections in several members of a family with the same organism occurred. Only 3 species, *S. typhi murium*, *S. choleræ suis* and *S. oranienberg*, offer sufficient instances for any generalizations whatsoever.

terium was recovered from 1 stool only. In interpreting the cultural findings, it must be realized that routine blood and urine cultures were not taken in most cases.

In general, there is a tendency for cer-

TABLE 1.—CLASSIFIED CLINICAL AND PATHOLOGIC DATA IN SPORADIC SALMONELLA INFECTIONS

	Total cases	Syndrome			Severity				Source of culture		
		G.I.	General	Both	Carrier	Mild	Severe	Death	Blood	Feces	Urine
S. typhi (*E. typhosa*)	19	1	9	6	3	2	13	1	11	19	1
S. cholera suis with var. Kunzendorf 2	11	0	6	5	0	0	7	4	8	1	2
S. typhi murium	20	3	3	9	5	5	5	5	2	19	1
S. oranienberg	16	3	0	2	8	3	2	0	1	12	0
S. derby	5	0	0	0	5	0	0	0	0	5	0
S. newport	5	1	0	2	1	3	0	1	0	4	0
S. urbana	2	2	0	0	0	1	0	1	0	2	0
S. schottmulleri (Para B)	2	0	0	2	0	0	2	0	0	2	0
S. give	2	1	0	1	0	0	2	0	0	2	0
S. montevideo	3	0	0	1	2	0	0	1	0	3	0
S. bareilly	1	1	0	0	0	1	0	0	0	1	0
S. london	1	1	0	0	0	1	0	0	0	1	0
S. thompson	1	0	0	1	0	0	1	0	0	1	0
S. oregon	1	0	0	1	0	0	1	0	0	1	0
S. panama	4	1	0	2	1	1	2	0	0	4	0
S. anatum	1	0	0	0	1	0	0	0	0	1	0
S. lichtfeld	1	0	0	1	0	0	0	1	0	1	0
S. morbificans bovis	6	4	0	0	2	4	0	0	0	6	0
S. muenchen	1	1	0	0	0	1	0	0	0	1	0
S. manhattan	1	0	0	0	1	0	0	0	0	1	0
S. newington	1	0	0	0	1	0	0	0	0	1	0
S. minnesota	1	1	0	0	0	1	0	0	0	1	0
Totals*	86	19	9	27	27	21	22	13	11	70	3

* Exclusive of *S. typhi*.

S. oranienberg presented the mildest syndromes, with 11 carriers giving no symptoms at all, and 3 presenting a mild picture with gastro-intestinal symptoms only. Two cases of *S. oranienberg* infection did present some toxic general manifestations, but there were no deaths. Most of the severest illnesses were found in cases of *S. choleræ suis*. All 11 of these cases showed toxic general manifestations, 5 with gastro-intestinal symptoms. There were 4 deaths, and the remaining cases were severe in this group. There were 8 cases of positive blood culture with the latter microörganism, while only 1 positive culture was obtained from the blood with the *S. oranienberg* infections. This microörganism was isolated in 12 instances from the stool, in contrast to the cases of *S. choleræ suis* infection, where the bacterium was recovered from 1 stool only. In interpreting the cultural findings, it must be realized that routine blood and urine cultures were not taken in most cases.

In general, there is a tendency for certain species to produce a more or less characteristic and typical syndrome. Allowing for some exceptions and variations, *S. choleræ suis* seems to cause the picture of sepsis with greatest uniformity, with and without "seeding foci" (Bornstein[3]). *S. oranienberg* shows a definite tendency to produce a milder clinical picture, or absence of all symptomatology in the form of the "carrier state." The gastro-intestinal picture with diarrhea or vomiting is the usual form that symptomatic infection with this organism assumes (Seligman, Saphra and Wassermann;[17] Sachs and Antine;[13] Jager and Lamb[4]).

S. typhi murium occupies a position in between *S. choleræ suis* and *S. oranienberg* in all of its manifestations and in its severity. In the 20 instances of *S. typhi murium* infection, the observed features of

the illness suggested an intermediary position from the standpoint of severity, the tendency to intestinal symptoms, and the tendency to invade the blood stream or to remain within the intestinal content. Thus, 5 of the 20 cases were asymptomatic carriers, only 3 presented general septic symptoms alone, while 9 showed gastrointestinal manifestations with accompanying general toxic symptoms. The microorganism was recovered from the feces in 19 cases, from the blood in 2 cases, and once from the urine.

Pathology. Nine of the 13 patients who died came to autopsy, representing the largest group as yet reported. The postmortem data will be found in Table 2. One death, not coming to autopsy, was a Medical Examiner's problem in a $2\frac{1}{2}$ year old child, admitted for extensive burns involving 63% of the body, who developed diarrhea after 3 weeks in the hospital, while apparently doing well. Blood culture yielded *S. typhi murium*. The patient developed a high temperature with convulsions, and died within a few days. This case may represent the activation of a latent carrier state or an infection acquired while on the ward, though a careful survey for potential carrier sources among the hospital personnel was unavailing. Of the 3 remaining patients who died and were not examined at autopsy, 1 had clinical pyelonephritis with *S. choleræ suis* in the urine; a second was an infant with congenital heart disease who developed an upper respiratory infection and diarrhea with *S. urbana* in the stool; and the last was a case of Wernicke's syndrome with advanced cirrhosis of the liver, in whom the infection may well have been a terminal blood stream invasion. The data from these unautopsied cases are given in Table 4.

TABLE 2.—AUTOPSY FINDINGS IN DEATHS WITH POSITIVE SALMONELLA CULTURES AT QUEENS GENERAL HOSPITAL

Case No.	Age	Culture	Source	Syndrome	Pathology and diagnosis
1—A-42-242	21	*S. typhi murium*	Stool, ulcers, peritoneum	G.I., acute appendicitis, operated	Typhoid-like ileocolitis with hyperplastic Peyers patches
2—A-41-489	33	*S. typhi murium*	Stool, blood, spleen, thigh, throat	G.I., diarrhea, chill with shock	Sepsis, with cellulitis of thigh; hyperplastic mesenteric nodes
3—A-45-218	18 mos.	*S. typhi murium*	Blood, lung, stool	Diarrhea, then convulsions, coma, high temperature	Hyperplastic Peyers patches mesenteric nodes, bacteremia
4—A-44-49	68	*S. cholera suis*	Kidney pus and blood	Cardiac and cerebral with coma (uremia?)	Suppurative pyelonephritis
5—A-43-477	9	*S. cholera suis*	Blood	Cerebral meningeal and then encephalic	Chr. cholangitis with biliary cirrhosis; hyperplastic Peyers patches
6—A-43-27	26	*S. cholera suis*	Stool	G.I. diarrhea with bleeding	Chr. active ulcerative colitis
7—A-44-135	46	*S. lichtfeld*	Stool, bile, stomach	G.I., typhoid-like	Marked gastritis and bronchopneumonia
8—A-44-226	60	*S. newport*	Pericardium	Chest pain—coma, diabetic	Suppurative pericarditis, cholangitis
9—A-41-455	$1\frac{1}{2}$ mos.	*S. montevideo*	Stool	G.I.—diarrhea	G.I. tract negative; bronchopneumonia and prematurity

TABLE 3.—AUTOPSY FINDINGS IN S. TYPHI MURIUM CASES ON FILE AT BETH ISRAEL SALMONELLA TYPING STATION

Case No.	Age	Sex	Source	Syndrome	Pathology
1	74	M	Spleen, bone	G.I., diarrhea, splenic tumor, severe anemia	Infected dissecting aneurysm of aorta with regional osteomyelitis, chronic cholangitis with cirrhosis
2	..	..	Stool	G.I., diarrhea	Acute arteritis, bronchopneumonia
3	66	M	Stool, from all path. sites	G.I.—severe diarrhea, vomiting, collapse	Pus filling gall bladder and in right ureter, petechiæ in ileum and sigmoid
4	49	F	Urine, spleen and intestinal wall	G.I.—outbreak of food poisoning with vomiting and diarrhea, uremia	Pus in urine, kidneys enlarged
5	5 wks.	F	Stool, blood	G.I.—diarrhea, vomiting	Thrombophlebitis with sepsis after intravenous
6	62	M	Blood and mitral valve	Fever only, no G.I.	Endocarditis
7	6 mos.	M	Blood (Pn. IV), then *Typhi murium*	G.I.—otitis with meningitis, then diarrhea	Possibly pneumonia ?
8	6 wks.	F	Stool and blood	G.I.—diarrhea, pneumonia	No data
9	50	F	Blood and spleen	G.I.—vomiting and diarrhea	No data

In general, the lesions found in the patients who came to autopsy presented a localization in the gastro-intestinal tract in 5 cases, with 2 instances of chronic cholangitis in addition (Cases 5 and 8); localization in the kidney in 1 case (Case 4); and involvement of the respiratory tract in 2 cases (Cases 3 and 6). It will be noted that 3 *S. typhi murium* cases came to autopsy, with a septic picture in 2 of them and a typhoid-like picture in 1 (Case 1, Figs. 1, 2, 3 and 4). In none of these 3 cases did there exist independent, obviously preëxisting pathologic lesions of sufficient magnitude to be significant in the mechanism of death. The Salmonella infections in the non-autopsied cases may well represent the insignificant incidental states, though the acute final episode suggests that the Salmonella infection was a lethal terminal event. Salmonella infection was the direct primary cause of death in all the cases coming to autopsy. This fact is significant of itself. In general, one is impressed by the mimicry of the different clinical forms and pathologic states of typhoid, and by the not infrequent severity of the infection.

Table 4.—Clinical Diagnoses in Salmonella Deaths Without Autopsy at Queens General Hospital

Case No.	Age	Culture	Source	Syndrome	Diagnosis
1—(L. W.)	48	*S. typhi murium*	Blood	Coma, peripheral neuritis	Cirrhosis of liver with Wernicke's syndrome
2—(M. P.)	2	*S. typhi murium*	Stool	Diarrhea; convulsions	Extensive first and second degree burns with diarrhea 3 wks. after admission
3—(K. G.)	69	*S. cholera suis*	Urine	Uremic and cardiac	Hypertensive heart disease; pyelonephritis (?)
4—(K. A.)	4 mos.	*S. urbana*	Stool	Bronchitis with diarrhea	Congenital cardiac with cyanosis

We studied the clinical course of 17 *S. typhi murium* deaths encountered by the National Salmonella Center at Beth Israel Hospital. The records of this group of cases were generously made available by Dr. Seligmann. The available findings in the 9 cases studied at autopsy are given in Table 3. For the entire 17 cases, a total of 14 positive cultures were obtained from the blood, 5 from the feces, 3 from the spleen, 2 from the urine, 2 from the intestinal wall, and 1 each from the liver, gall bladder and mitral valve. The septic picture was again prominent. A further analytical discussion of this group would be repetitious. Only 2 of the cases showed general symptoms of sepsis only. The remaining 15 cases showed some gastro-intestinal symptoms along with severe general toxic manifestations. Others showed gastro-intestinal symptoms only, but this would hardly be expected often, since the group is a select one of cases severe unto death.

The first autopsy of this series (Table 2) was performed by Dr. N. Mitchell in 1941 on a patient who was operated upon for acute appendicitis and died of peritonitis. At autopsy, this patient showed the typical morphologic picture of typhoid fever (Figs. 1, 2, 3 and 4). The microorganism cultured from several sites was *S. typhi murium*. A few months later, another patient was admitted with a clinical picture of typhoid fever, and from his blood stream *S. typhi murium* was also isolated. At autopsy, this second case failed to show the characteristic hyperplastic lesions in Peyer's patches, though the mesenteric lymph nodes were hyperplastic (Case 2, Fig. 5). In a third case, that of an 18 month old child who was thought to have encephalitis because of coma and convulsions, hyperplastic Peyer's patches and mesenteric nodes, without ulceration of the gastro-intestinal tract, were found. The other instance in which hyperplastic Peyer's patches were found at autopsy represented an infection with *S. cholera suis*. The patient was a 9 year old child with chronic cholangitis with biliary cirrhosis. In 1 case in which a clinical diagnosis of typhoid fever was made, there was only marked gastritis (Case 7, Fig. 6), with no changes in

FIG. 1.—Case 1. The cecum and small intestine showing hemorrhagic hyperplastic Peyer's patches and lymphoid tissue and superficial ulcers of ileum and cecum. (Approx. × ½.)

FIG. 2.—Case 1. Minute ulceration of large bowel showing fibrin and predominant mononuclear exudate. (Low power; approx. × 40.)

Peyer's patches; *S. lichtfeld* was isolated. The remaining autopsies showed no morphologic alteration in the gastro-intestinal tract (Table 2).

Autopsy disclosed only 2 cases with hyperplasia of Peyer's patches (Cases 1 and 3), identical to that seen in typhoid fever. Study of the autopsied cases, however, emphasizes the frequency of blood stream invasion and sepsis in the fatal cases. In the few cases where a routine blood culture was taken clinically in this group, the significant microörganisms were found. One case showed ulcerative colitis. In general, these, as well as other morphologic lesions which were found, all speak for bacteremia. The septic clinical picture associated with the typhoid-like syndrome encountered in Cases 1, 2, 8 and with the respiratory tract involvement in Cases 8, 10, 11 and 12, all favor an existing bacteremia, despite the ab-

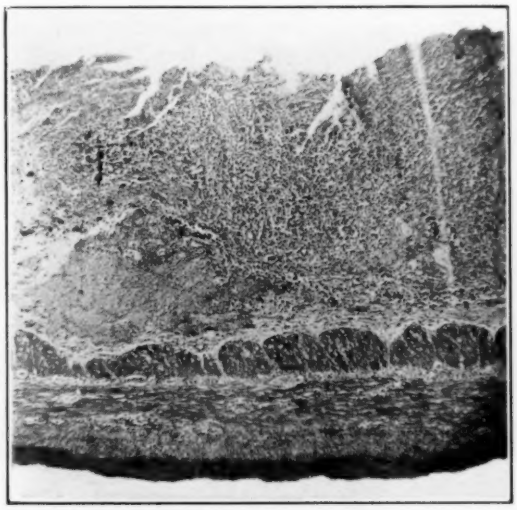

FIG 3.—Case 1. Region of Peyer's patch showing prominent mononuclear exudate and fibrin. (Medium power; approx. × 40.)

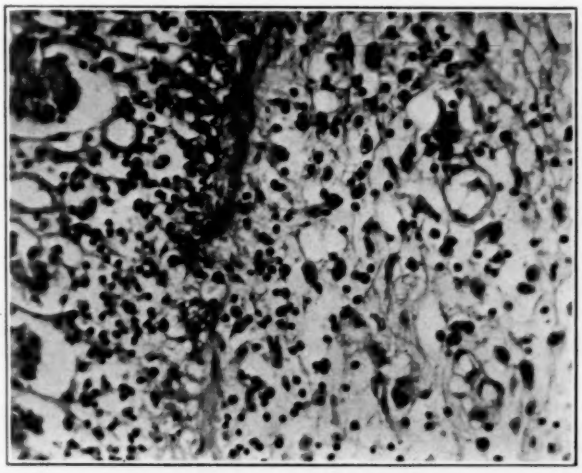

FIG. 4.—Case 1. Higher magnification showing characteristic mononuclear exudate. (High power; approx. × 320.)

sence of proof by positive blood cultures in many instances. Such bacteremias are known to occur quite regularly in cases of typhoid and pneumonia.

Most of the severe and nearly all of the fatal cases presented a septic picture with bacteremia as shown by blood culture. The fatal cases in which blood stream invasion was not demonstrable represented sudden deaths with inadequate study, young children or newborn nursery victims. The tendency to belittle Salmonella infections is not justified by the clinical course often seen in the very young. In Case 8, there was a localization of such a blood stream infection in the pericardium (Fig. 7), and a cholangitis, with *S. newport* recovered from this site. This, of course, implies a passive blood stream invasion, with the biliary tract as a seeding focus in this instance.

The known blood stream invasion in

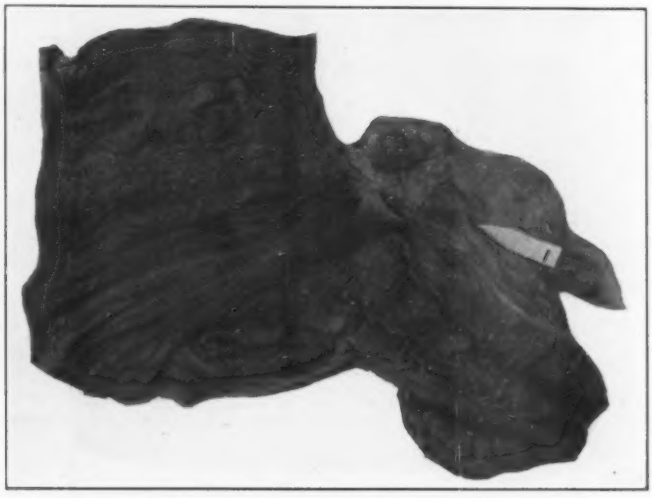

FIG. 5.—Case 2. Ileocecal region showing hyperplastic hemorrhagic mesenteric nodes and congested mucosa. (Approx. × ½.)

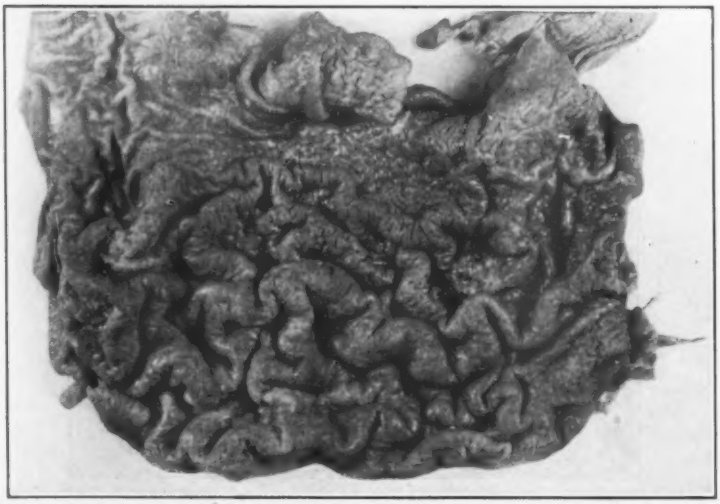

FIG. 6.—Case 7. Stomach showing marked acute diffuse gastritis. (Approx. × ½.)

experimental Salmonella infection studies, and its independence of the portal of entry, parallels these observations. In the experimental animals, bronchitis and bronchopneumonia and cholangitis are very common. The pathogenesis of the enteric lesions as indicated by the experimental work of Orskov and co-workers,[8,9,10] Seiffert and co-workers,[16] Takita,[18] Waldman,[19,20] and Müller[6] renders the variable localization and mutable clinical picture explicable. Much of the experimental work was carried out with *S. typhi murium* in mice. It was established that the microörganism very rapidly invaded the blood stream, to be excreted in the bile. The lesions in the gastro-intestinal tract, with the localization in lymphoid nodules and Peyer's patches, really represent secondary lesions, rather than a primary portal of entry as first considered. It is this period of early or immediate generalization, with its immunologic significance, that gives the opportunities for the variations encountered.

In a report on experimental studies in animals, to be presented in detail elsewhere, nearly all lesions seen in the human autopsy series were encountered. The typhoid lesions in the gut could be produced with regularity by controlling dosage and method of administration. The cholangitis, and the lymph node and spleen involvement were common. The kidney localization did not seem to occur, though others have noted this finding.

The only instance in the literature in which the typhoid-like morphology of the Salmonella lesions in a human case was adequately described was reported by Neesen and Merkel,[7] who found 1 such case in a group of 4 deaths due to paratyphoid Breslau (old terminology for *S. typhi murium*).

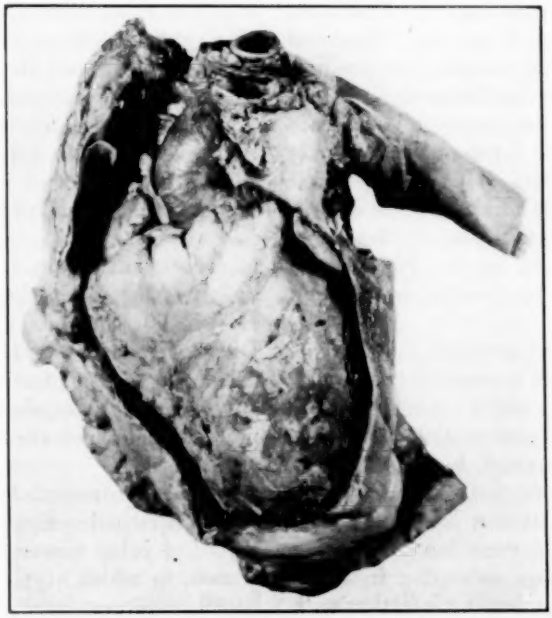

FIG. 7.—Case 8. Showing fibrinopurulent pericarditis. (Approx. × ¼.)

It was felt that this tendency to localization in the Peyer's patches in the intestine might be accountable on the basis of strain variation. To test this hypothesis, several strains of *S. typhi murium* were fed to laboratory animals: at first guinea pigs, rats and mice, and then guinea pigs and mice only. In all, 6 strains of *S. typhi*

murium were used for this purpose (Grier, Thomas, 1182 B.I., Kaufman, B.I. 43930 N.Y.S. lab., Williams). The initial group of guinea pigs fed the strain of microorganisms recovered from this initial case (Grier) showed remarkable localization of involvement in the region of Peyer's patches and mesenteric lymph nodes, in contrast to some of the other strains used for comparison (43930, Williams). The findings in the remaining viscera are not being considered here but showed the corresponding visceral typhoid-like lesions. These original observations have not been corroborated and have been corrected by subsequent experiments in guinea pigs, which showed that several other Salmonella strains (*S. typhi murium*, Kaufman B.I., enteritidis and Thomas) can produce such lesions. The detailed analysis of our failure to correlate serologic antigenic structure with the form of reaction will be given separately. It may be stated that the method of administration is most important, and that placing favorable doses of organisms in the food favors localization of the lesions in Peyer's patches.

Biology. Several members in the Salmonella group show a remarkable selective specificity for particular hosts while others, though they have preferential hosts, may also infect other species, but usually producing a milder infection. A microörganism like *S. typhi murium* has a wide host range with a wide variation in reaction and symptomatology extending from the carrier state to fulminating infection with death. This bacterium has become most widespread in nature with the widest range of pathogenic adaptability to multiple hosts. The phenomena of variation and adaptation for bacteria are best studied in this group (Savage[15]). The general intermediary position of *S. typhi murium* infections between the septic picture of *S. cholera suis* and the enteric picture of *S. oranienberg* was obvious in our own clinical material by the numerical incidence of the characteristic clinical features, and from the standpoint of general clinical impression, as well as by the morbid changes and the laboratory data. Our experimental studies confirm the ready adaptation of this organism for the study of the multifarious forms of reaction of the enteric group.

The members of Salmonella group show interesting serologic relationships to known pathogens such as *E. typhosa* as well as to variants of the originally non-pathogenic *B. coli* group (Saphra and Silberberg[14]). A complete analysis of our own unavailing attempts to conciliate morphologic changes produced by the Salmonella on the basis of serologic analysis will be recorded elsewhere. This is in keeping with the experience of others: Boivin,[1,2] Pike and MacKenzie,[11] and Pike and Swinney,[12] and with the expressed opinion of Seligmann and Saphra.

The biochemical features showing gradation and transition between the Salmonella group are better known. We have encountered several gasless *S. typhi murium* strains. The original identification in each instance was typhoid by us and by the New York City Department of Health, but complete antigenic study by Dr. Seligmann and Dr. Saphra established the correct identification as a variant of *S. typhi murium* which failed to produce gas under the normal controlled conditions.

We wish to record 2 cases with severe gastro-intestinal symptoms, clinically suspected of being caused by Salmonella infection, in which atypical forms of colon bacilli were recovered. In the first instance, a pathogenic rôle is suggestive; in the second case, the ulcerative lesions are often found above an obstruction. This probably represents a common finding and the atypical coli form of bacillus may be a mere concidence.

Clinical Notes. Case A–45–34: The patient was admitted with an acute abdominal episode and considered to be mesenteric thrombosis or preëxisting duodenal ulcer. Severe epigastric pain was the outstanding finding. At autopsy, severe gastritis and enteritis were found. Only an atypical coli

organism was recovered, present in great numbers.

CASE A–40–58: A 65 year old female admitted with intestinal obstruction died with an episode of acute dyspnea and abdominal pain. The autopsy showed extensive deep ulcerative lesions of the colon above an obstructing carcinoma. Bacteriologic study revealed an atypical coli organism and a streptococcus non-hemolyticus.

We do not mean to imply that an atypical colon bacillus is invariably a pathogenic microörganism. Others have suggested that this bacterium can produce symptoms and lesions. The existence of transitional biochemical and serologic phenomena between atypical colon bacilli and the Salmonella group might lead us to expect pathogenic strains of atypical *B. coli* to occur on theoretical grounds.

We attempted studies of the pathogenicity of the atypical Coli organisms recovered from these 2 cases in guinea pigs and mice by feeding experiments, but obtained no evidence of infection in the form of any lesions or deaths. We do suspect that transition forms of *B. coli* will be found which will be pathogenic for some humans and animals on ingestion.

Epidemiology. Though all of the material represents sporadic cases, several interesting epidemiologic observations were encountered in this survey. Several double infections were encountered. Two nurses with *B. oranienberg* showed, in addition, a superimposed *S. manhattan* and *S. typhi murium* respectively. One baby showed a double infection with *S. morbificans bovis* and *S. muenchen*.

Some of the types encountered, as *S. morbificans bovis*, *S. panama* and *S. lichtfeld*, are distinctly rare. Our attempts at epidemiologic analysis were disappointing. At one time we had 6 premature infants showing *S. morbificans bovis* in their stools. Three of these infants showed no symptoms whatsoever. The other 3 showed mild diarrhea. None was severely ill. No source for this epidemic could be established in the hospital staff on the maternity ward or in the dietary division.

One patient was admitted with vomiting, high fever and diarrhea, and *S. typhi murium* was obtained in the stool. The patient was discharged as cured after 4 weeks of illness. The sister of this child was admitted 4 days after the onset of her sister's symptoms for an incidental surgical repair of an umbilical hernia. A stool culture showed this same organism, *S. typhi murium* but no symptoms were present and no history relative to such a previous infection could be obtained.

One student nurse developed a diarrhea, and *S. oranienberg* was recovered. The patient was discharged within 3 days. A survey showed that a waitress in the nurses' dining room had this organism in her stool. This waitress admitted a bout of diarrhea a short time previously.

Summary and Conclusions. During a 4 year period at the Queens General Hospital, 86 sporadic instances of Salmonellosis, revealing 21 known serologically identifiable strains were encountered. The study includes a tabular correlation of the nature and severity of the clinical picture, and the bacteriologic data, with particular reference to the sources of the cultures.

The 3 most common species, *S. cholera suis*, *S. typhi murium* and *S. oranienberg*, are compared. The carrier state, the enteric fever form with and without gastro-intestinal manifestations, and the septic forms alone and in combination, are presented from the standpoint of their individual features and their inter-relationships. The syndromes presented by the 13 cases who died, and the pathologic findings in the 9 instances examined at autopsy, are tabulated, with a correlation attempted with the form of the clinical illness, the bacteriologic data and the morbid changes encountered.

The intermediary position of *S. typhi murium* in this comparison, from the standpoint of the clinical picture, the severity of the illness and the morphology of the lesions, is stressed, and the biologic implications noted. The data from 17 *S. typhi murium* deaths on file at the Beth Israel Hospital Salmonella Typing

Center and the autopsy findings in 9 of them are tabulated for comparison.

The problem of the adaptation of the Salmonella organisms to different hosts, and the variable course the infection pursues in the different and in the same hosts, is discussed. The variation in the clinical course is stressed. Reference to the lesions of experimental Salmonella infection under varying conditions, appearing in a separate communication, is included for comparison with the human morbid pathology. An analysis of the clinical course and the pathologic data is presented in the light of experimental studies of the pathogenesis of the enteric disease group, and the resemblance to typhoid fever in its mutable and multifarious forms. Typhoid-like lesions at autopsy caused by *S. typhi murium* are emphasized. The broad biologic problem of Salmonellosis is presented from the standpoint of its relationship to the atypical coli infections of the gastro-intestinal tract on the one hand, with 2 instances cited, and with typhoid infections on the other.

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HOOKWORM INFECTIONS IN TROOPS RETURNING FROM THE PACIFIC

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Routine stool surveys of men returning to this country from various Pacific Islands have disclosed, among other parasites, a large number of hookworm infections. Of 2500 consecutive admissions to this hospital from the Pacific Theatre, 11.5% were found to have hookworm eggs in the stool. By contrast, the incidence of hookworm infestation in 4300 patients who had seen only continental service in this country was 6.2%.* The composition of both groups from the standpoint of residence in southern hookworm states is sufficiently uniform so that this factor is not responsible for the difference observed. The complete details of those surveys are being reported in another paper (Lt. Col. T. B. Wilson, M.C., to be published).

The purpose of this paper is to present observations on the nature, severity and results of treatment in patients with hookworm infection who had served in the Pacific Theatre and who had been evacuated for other reasons.

Material and Methods. This report deals with 284 patients. In 210 the diagnosis of hookworm infection had been made overseas while in 74, eggs were first demonstrated at this hospital. The complete clinical records of the patients in whom hookworm eggs had been found in the stools overseas or in other hospitals in this country were reviewed to determine the total number of treatments they had received prior to admission to our Tropical Disease Section. If hookworm eggs were found on admission, all previous treatments were considered failures, but if the stools were consistently negative the last treatment for a positive stool was considered successful. All patients in this series with positive stools at this hospital received one or more courses of treatment, not on clinical grounds, but to determine if possible the genus of the hookworm which they harbored as well as the efficacy of treatment.

Each stool was examined at least once by direct smear, zinc sulfate centrifugal flotation, brine flotation, and water centrifugal sedimentation. Stoll egg counts were done before treatment in 75 patients. The routine treatment employed was as follows: 30 gm. of sodium sulfate were given after the evening meal the night before treatment. No breakfast was allowed, and 3 cc. (occasionally 5 cc.) of tetrachlorethylene given in soft capsules. Two hours later another 30 gm. of sodium sulfate were given. Following treatment, all stools were collected directly into a large wide-mouthed jar until 4 P.M. of the day of treatment. The stool was then washed through a metal sieve and if adults were recovered, they were identified by the morphology of the mouth parts. The total number, genus and sexes were recorded.

Results. 1. *Severity of Infection.* The average Stoll count on admission in 17 patients who had been treated on 1 or more occasions overseas and subsequently were found here to have Necator infections was 1900 eggs per gm. of feces, with a range of from 100 to 9100. The average Stoll count on admission in 28 patients who had been treated on 1 or more occasions overseas and subsequently were found here to have Ancylostoma infections was 2295 eggs per gm., with a range

* This figure is, however, not to be regarded as representative of the nation-wide picture, for there is evidence to suggest that men from northern sections acquired their infection while in southern training stations.—The Editors.

of from 200 to 9800. These findings may not reflect the original degree of infection, since all these patients had had 1 or more courses of treatment prior to admission here. However, the average Stoll counts prior to treatment in 15 patients in whom eggs were first discovered at this hospital and who subsequently were shown to harbor Necator or Ancylostoma were 2400 and 3200, respectively. This, as well as the total number of worms recovered subsequently (see below) indicates that the hookworm infections acquired overseas in the majority of patients were very light.

2. *Adult Recoveries.* Following treatment, adult hookworms were recovered from 169 patients. The ratio of female to male for Necator was 1.6 and for Ancylostoma 2.3. The total number of worms as well as the genus recovered from these patients is summarized in Table 1.

Table 1.—Recovery of Adult Hookworms Following Treatment With Tetrachlorethylene in 169 Patients

	No. patients	No. of adults recovered					
		1–5	6–10	11–25	26–50	51–100	101–150
Necator	69	41	12	10	6		
Ancylostoma	87	56	15	10	4	2	
Mixed	13	4	3	4	..	..	2
Total	169	101	30	24	10	2	2

The maximum number of Necator was 86 and of Ancylostoma 112. In only 14 of the 169 patients from whom adults were recovered were more than 25 worms found. Following treatment of 36 patients no adults were recovered although repeated stool examinations subsequently were negative for eggs. Failure to find adults in these patients who were apparently cured of the infection is regarded as evidence that the number of worms they harbored was very small, although it is admitted that the duration of collection may not have been long enough to find them. This also applies to 79 patients from whom no adults were recovered after treatment, although subsequent stools were positive, indicating treatment failure. Thus, on the basis of the total number of worms recovered following treatment as well as the Stoll egg counts, it seems evident that the hookworm infections in the majority of these men are relatively light.

It is important to note that 65% of the patients from whom adults were recovered harbored Ancylostoma. Only 12% of the latter resided in southern states or had received military training in possible hookworm areas in this country. On the other hand, 75% of the men with Necator infections resided in southern states in this country. It seems probable, therefore, that the increased incidence of hookworm among men returning from the Pacific is due largely to the acquisition of Ancylostoma infections overseas and that the ratio of Ancylostoma to Necator acquired in the Pacific is at least 3:1.

3. *Efficacy of Treatment.* Tetrachlorethylene has been reported as producing cures in 70 to 90% of patients with hookworm infections following a single course of treatment with this drug. In our experience the results of treatment judged by the number of courses administered to the same patients overseas who still had positive stools on admission here are not nearly as encouraging. Of 100 patients who received one or more courses of tetrachlorethylene overseas, only 42% were cured of their infection after the first course of treatment as determined by repeated stool examinations at this hospital. The results of multiple treatment overseas in these patients whose records were complete are presented in Table 2.

Since no adult recoveries were done following these treatments, no conclusions can be drawn concerning the relative efficiency of tetrachlorethylene in eliminating Necator *versus* Ancylostoma infec-

tions. However, since 65% of adults recovered from 169 patients were Ancylostoma, the relatively poor results of multiple treatment overseas may be due to the persistence of this parasite. In this connection, it is of interest that of 35 patients in whom eggs were first discovered at this hospital, and who were treated for the first time for hookworm infections which were subsequently proved to be Ancylostoma, only 9 (25%) were cured after the first course of drug; 18 patients were re-treated and 7 (39%) were cured, making a total of 55% cured after 2 courses of treatment. On the other hand, of 24 patients diagnosed and treated for the first time at this hospital for infections subsequently proved to be Necator, 16 (66%) were cured. Three out of 5 who were re-treated were cured, or a total of 85% cured after 2 courses of treatment. These observations, while limited in number, suggest that multiple treatments overseas because of repeatedly positive stools were the result of Ancylostoma infections in the majority of such patients and that tetrachlorethylene is less effective against Ancylostoma than Necator. The relatively low incidence of double infections with Necator and Ancylostoma in returnees from the Pacific (13 cases in 169 patients) may be due not only to a lower incidence of Necator in some Pacific areas but to elimination of Necator from a large proportion of the men as a result of several courses of treatment prior to their return to this country.

4. *Other Parasites.* Hookworm stools frequently had additional parasites. Of 197 patients who had hookworm eggs in the stools, 80 had 1 or more helminths, 59 had 1 or more protozoa, and 22 had both. The incidence of *E. histolytica* in this group was 11%. The occurrence of *S. japonicum* is a reflection of the fact that many of the patients in the group had served on Leyte and had been returned because of proven or suspected schistosomiasis; the figure shown, therefore, cannot be taken as indicating the incidence of this infection in the Pacific. The distribution of parasites other than hookworm in this group is shown in Table 3.

5. *Clinical Aspects.* The clinical records of 100 malaria and surgical patients with hookworm as the only helminthic infection were reviewed. In no case were any signs or symptoms found which could be attributed to hookworm infeetion alone. The average red cell count was 4.2 million per c.mm., the lowest being 3.3 million. Only 10 patients had less than 4 million red cells and in these the anemia appeared to be related to a recent attack of vivax malaria or a chronic surgical infection rather than hookworm infection. The average percentage of eosinophils in this group was 10.2. In our experience hook-

TABLE 2.—EFFICIENCY OF REPEATED COURSES OF TETRACHLORETHYLENE ADMINISTERED OVERSEAS IN 100 RANDOM PATIENTS WITH HOOKWORM INFECTIONS

Based on Complete Clinical Records and Stool Examinations on Admission—Includes Ancylostoma, Necator and Mixed Infections

No. treatments	No. treated	Subsequent stools		
		Positive	Negative	Total % cured
1	100	58	42	42.0
2	48	34	14	62.2
3	30	18	12	79.1
4	16	12	4	85.7
5	8	4	4	95.0
6	3	1	2	98.7

TABLE 3.—DISTRIBUTION OF OTHER PARASITES IN 197 PATIENTS WITH HOOKWORM INFECTIONS

	No.	%		No.	%
E. histolytica	22	11	*S. japonicum*	68	34
E. nana	41	20	*T. trichiurus*	26	13
E. coli	21	10	*S. stercoralis*	7	4
I. bütschlii	11	5	*A. lumbricoides*	4	2
G. lamblia	8	4			

worm infection in service men returning from overseas are not clinical and do not represent hookworm disease.

Summary and Conclusions. 1. The incidence of hookworm infection in troops returning from the Pacific is 1.8 times that found in troops who have served only in this country.

2. Of 169 infections in which the type of hookworm was determined, 65% were due to Ancylostoma. The increased incidence of hookworm infections found in service men returning from various Pacific islands is believed largely due to the acquisition of Ancylostoma overseas.

3. In our experience tetrachlorethylene is not as efficient in eliminating Ancylostoma as it is in Necator infections, and this may account for persistently positive stools after multiple courses of treatment overseas.

4. The demonstration of a high proportion of Ancylostoma infections in men returning from the Pacific may be of concern to public health authorities in this country, in view of the possibility of the introduction and establishment of this parasite.

5. Hookworm infections in the majority of returning servicemen are light and rarely represent hookworm disease.

6. Multiple parasitism is common in men returning from the Pacific. Finding 1 or more parasites, though these may be non-pathogenic, should stimulate continued search so that potentially serious infections are not overlooked.

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FIBROCYSTIC DISEASE OF THE PANCREAS

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IT is unfortunate that the name, fibrocystic disease of the pancreas, is a purely descriptive pathological term, the use of which is necessitated by our lack of knowledge regarding the etiology of the disease. In the period prior to 1938, individual cases were reported under many and varying descriptive terms. The synonyms are as follows: pancreatitis, pancreatic disease, atrophy of the pancreas, congenital pancreatic steatorrhea, celiac disease, congenital cystic fibromatosis of the pancreas, agenesis of the exocrine portion of the pancreas, congenital pancreatic disease, cirrhosis of the pancreas, congenital familial steatorrhea, chronic interstitial pancreatitis in infancy and pancreatic insufficiency (Wolman).[73a] As is readily seen, many of these terms are of clinical origin and arose during the twenty odd years before the appearance of Dorothy Andersen's classical article in 1938,[1a] clarifying and correlating the clinico-pathological relationships. As will be understood, when the pathological findings and etiological factors are discussed, the name fibrocystic disease is not satisfactory but a more adequate one is lacking. Although the cysts are mainly microscopic, it seems unwise to call it simply fibrosis of the pancreas as the latter appears to be secondary to the acinar lesions. Besides, fibrosis is a marked feature of congenital syphilis (Porter[54]), and this term might be confusing.

The clinical, biochemical and pathologic findings are closely inter-related in this disease and to reach an understanding of the pathogenesis and etiology, it will be necessary to touch briefly on the clinical and biochemical aspects. But within the space of this review, it will be impossible to enter into all of the clinical details which may be found in other publications referred to below in their appropriate places.

It must be noted before proceeding further that the pancreatic changes give rise to two clinical entities:[1a] meconium ileus of the newborn and the syndrome of pancreatic steatorrhea in later life. In the former, the pancreatic changes are well advanced at birth, while in the latter, the changes are slowly progressive over months or years.

HISTORICAL. It is of interest to sketch

the historical background of the disease as it illustrates the long period that may elapse, in a relatively common disease with typical pathological findings, before the point of supersaturation is reached and a single observer gathers the evidence together and crystallizes the clinical and pathological findings into a disease entity. Prior to Andersen's paper,[1a] published in 1938, about 27 cases had been reported under a variety of names. In the 8 years elapsing since 1938 several hundred have been reported. Furthermore, Andersen's article has stimulated a renewed interest in the exocrine pancreatic secretions which had been previously over-shadowed by its endocrine function. This was in part due to the difficulty of estimating pancreatic enzymes, of interpreting any changes noted and lastly to the fact that adult pancreases do not commonly show lesions of the exocrine portion of the gland, except in association with obstruction of the pancreatic ducts by stones or carcinoma or in acute pancreatic necrosis.[4,11a,47,60,61]

Much of the delay in establishing this disease as an entity was caused by writers clinically regarding these cases as Gee-Herter's (celiac) disease and the apparent failure of observers to note the isolated case reports appearing in the literature of proven advanced pancreatic disease with clinical symptoms of celiac disease. Parsons,[50] in his Rachford lectures in 1932, stated that the pancreas is normal in celiac disease, which is, of course, true, but he makes no mention of the existence of pancreatic deficiency disease. Undoubtedly some clinicians were on the right track but failed to report adequately such pathological material as they obtained or interpreted the findings purely as secondary lesions.[55,67] Some cases were buried in the literature on vitamin A.[7,72]

Undoubtedly, the pathologist was at fault in not taking routine autopsy sections of that unassuming organ, the pancreas. In a marasmic infant or a child with bronchopneumonia, there would appear to be sufficient cause of death, without taking sections of all organs. Furthermore, because many of these infants die under 1 year of age, the clinician failed to note the intestinal symptoms, so frequently overshadowed by the respiratory disease. The paucity of cases reported prior to 1938 would seem to be accounted for in this manner. Awareness of the disease has markedly increased its reported incidence.

The pancreatic lesions were probably first described by Landsteiner[42] in 1905 but his was a case of meconium ileus and probably, therefore, was ignored. Between 1905 and 1919 several suggestive clinical cases were published but without post-mortem examination.[8] These were followed by isolated reports with pathologic studies in the literature up until 1937.[5,7,12,13,15,23,28,30,32a,33,35,41,49,64,69,72]

Although Parmelee[49] separated his cases from true celiac disease and apparently had a clear understanding of his cases, they were too few in number to make an impression on the pediatric world. The same probably applies to Harper's report in 1930[32a] and 1938.[32b] Finally, in 1938, Andersen[1a] clarified the status of the disease in a report on the findings in 22 new cases plus the above 27 cases and gave a clear description of the clinical and pathological aspects. In the same year Harper,[32b] Thomas and Schlutz,[68] and Blackfan and May,[6] in a series of independent observations, also confirmed Andersen's conclusions.

Since 1938 numerous cases[2,16,18,24c,26,27,-34,45,52,58,59] have been reported but relatively little or no advance has been made on the problem of etiology. Improved techniques for obtaining and estimating the pancreatic enzymes, resulting in more accurate clinical diagnoses, have constituted the main advance. Treatment of the disease, studies of stool and blood lipids, detailed pathologic anatomy and one attempt to reproduce the disease, have been the principal subjects of investigation.

Clinical Aspects. The disease clinically can be almost identical, in symptomatology, with idiopathic celiac disease so far as the gastro-intestinal system is concerned, the main differences being in

the earlier age of onset and the high incidence of suppurative lung lesions and the fatal outcome in fibrocystic disease of the pancreas.

In most cases the time of onset is at birth or within the first 6 months to 1 year of life.[1a,52] Although the severity of the pathologic changes in the pancreas vary considerably in different cases, no attempt to correlate the age of onset or severity of symptoms with the pancreatic changes has been made. The age at death varies somewhat in the series reported but ranges from 2 days to 14½ years.[1a,6,52] About 70% of patients die during the first year of life, mainly as the result of respiratory disease. A small percentage survive beyond this period but the eventual outcome is fatal.

The symptoms are marked by their variability but the cases may be divided into: (1) those with predominant respiratory symptoms; (2) those with predominant gastro-intestinal symptoms, and (3) those with a combination of both respiratory and intestinal disturbances.[52]

Andersen[1a] divided her cases in 3 groups and this seems to have been followed by subsequent observers. These are: Group 1—patients dying in the neonatal period, usually with meconium ileus with absence of other gastro-intestinal symptoms or lung infection. Group 2—patients dying within the first 6 months of life (later observers have extended this group to 1 year) and having nutritional disturbances often obscured by respiratory symptoms. Group 3—patients dying after 1 year of age with gastro-intestinal symptoms resembling celiac disease.

Andersen's second and third groups are characterized by respiratory symptoms, emaciation and under-development in spite of an excellent appetite. The stools may appear to be normal in early infancy, in many cases, but with the giving of solid foods the stools become large.[1a,1e] The variation in the description of the stools by different writers is probably due to the effects of multiple factors influencing digestion and absorption, which vary from patient to patient.

All cases give a history of cough, sooner or later; the onset varying from 2 weeks[1a] to upwards of 11 years.[49] It progressively becomes more severe and eventually patients develop purulent bronchitis, bronchiectasis and pneumonia.

INCIDENCE. The incidence of this disease, as found at autopsy in children, varies somewhat from author to author. Andersen[1a] reported an incidence of 3.3% in 605 autopsies, Menten and Middleton[45] reported an incidence of 2.8% between 1936 and 1943 but could find no cases in their autopsy material for the 10 years prior to 1936. Blackfan and May[6] reported 1.3% and Farber[24b] 4.8% between 1937 and 1942. The latter author found an incidence of 12% in 1942. Some authors[45,65] consider that the disease is more prevalent than formerly. Such opinions do not seem justifiable at the present time as more and more cases with less marked lesions are being diagnosed. For example, Andersen[1a] reported only those cases with 90% or more of functionless pancreatic tissue yet states that 11.4% of the pancreases examined by her showed less marked lesions. One may conclude that the true incidence is not yet established but may be in the region of 4% or more of autopsies.

Andersen[1a] gave the sex incidence in her series as about 67% females and 33% males, suggesting that females were more frequently affected. Since then, it has become apparent that there is no sex predominance in fibrocystic disease of the pancreas.[52]

The familial incidence is relatively high as reported by Howard[34] in 1944. He reviewed 120 cases and found 12 families in which each of 2 children had had the disease. Several authors have reported the disease as occurring in twins, sometimes affecting only one twin,[12,27,58] at other times both twins.[6,7] None of the twins appeared to be identical. No studies have been done with reference to the Mendelian principles.

RACE AND DISTRIBUTION. Cases have been reported from Australia,[32b] Canada,[38,65] England,[13] Germany,[5,42,51,64,69] Hol-

land,[35] New Zealand,[18] and the United States. The parents may come of Italian, German, Irish, English, Scotch and American stocks.[1a,45] Whites would seem to predominate as Andersen reports only 1[1a] and Menten and Middleton[45] only 2 cases occurring in Negroes.

ROENTGENOLOGICAL ASPECTS. The features of this diagnostic aspect have recently been reviewed by Pugh[57] in this journal. Roentgenologic studies indicate that there are non-specific intestinal changes and that the bilateral, widespread lung changes are suggestive but not pathognomonic of fibrocystic disease.

PATHOLOGIC ANATOMY. Externally the body is characteristically underdeveloped and emaciated with gross evidence of muscular atrophy. The abdomen is often protuberant and there is frequently an umbilical hernia. The body cavities are usually normal except that the pleural cavities may show evidence of inflammation, as would be expected from the incidence of lung lesions.

PANCREAS. The pathognomonic lesions essential to pathologic diagnosis are found in the pancreas and it is on the basis of the pancreatic changes that the disease entity has been distinguished from others. It must be emphasized that so far as can be judged from the histopathology of the pancreas, the lesion is essentially one of acini and ductules. The fibrosis is a secondary phenomenon upon which too much emphasis has been laid and which unfortunately is included in the cognomen of the disease for lack of a better name. This fact has been pointed out by Farber[24c] but needs to be re-emphasized. The secondary character of the fibrosis is clearly evident from the appearance of the milder lesions in which there may be little or no fibrosis but definite acinar-duct changes. Thus the lesion is essentially one of the exocrine portion of the gland.

Grossly, the organ may or may not show changes,[1a,24c] depending on the extent of the lesion and possibly also on the duration. Farber[24c] has given the most extensive pathologic report on the pancreas with 87 cases as material. As Andersen[1a] has stated, accurate gross diagnosis is markedly improved by experience. The pancreas is usually firmer, smaller and thinner than normal. The weight is less than normal, although it must be remembered that there is a wide variation in normal weights. Mitchell and Nelson[46] give the following normal values: Newborn and up to about 2 months of age, 2 to 3.5 gm., 1 year of age, about 10 gm., 4 to 5 years, 20 gm., and 10 to 12 years, 30 gm.

In those cases with advanced fibrosis there may be a distinct grittiness on cutting into the pancreas. If there is marked cyst formation and advanced fibrosis, the organ will present an irregular furrowed appearance due to alternating depressed fibrotic areas and elevated cystic or uninvolved normal pancreatic tissue. The cut surface will exhibit irregular lobulation with intersecting wide bands of connective tissue, as opposed to the regular, normal diamond-shaped pattern with scarcely visible septa. In some cases the cut surface, particularly in the head of the gland, will show dilated ducts filled with yellow plugs. Cysts visible to the naked eye are not often present. Occasionally the pancreas is grossly fatty.

The patency of the main pancreatic ducts is difficult to ascertain with any degree of accuracy but the consensus is that they are patent in the majority of patients living beyond the neonatal period. The incidence of atresia in meconium ileus is higher. It is extremely difficult to dissect the main ducts in infancy due to their small size. Farber[24c] states that in his series, a few cases showed definite narrowing of the ducts but in the majority no congenital atresia or stenosis was found. There was stenosis in Kornblith and Otani's case[41] and in Hurwitt and Arnheim's[38] as proven by following the duct of Wirsung in serial sections. Cases of stenosis are reported by Benoit[5] and Tiling.[69] In Andersen's[1a] own cases attempts to demonstrate the patency of the ducts were unsuccessful in 4 out of 5 cases;

the lumen appeared to end in fibrous tissue 5 to 15 mm. from the ampulla of Vater. In the other case the pancreatic duct was dilated throughout.

An important argument in favor of patency of the ducts is put forward by Farber.[24c] A history of meconium ileus is not given by those patients who survive the newborn period. If congenital obstruction of the ducts were the cause of pancreatic fibrosis, then meconium ileus should be a constant finding. This has not proved to be the case. However, Oppenheimer's case[48] would seem to contradict this statement, as duct atresia was found in a 10 months' old infant who had no history of meconium ileus.

Microscopically, the essential lesion is accumulation of an eosinophilic secretion in the acini and small and large ducts. The eosinophilic material is homogeneous and frequently lamellated, more particularly in the larger accumulations. Some of the larger masses contain fragments of eosinophilic substance mixed with pale faintly yellow material. This material does not give the staining reaction of mucus by mucicarmin methods[71] but on the other hand, Baggenstoss and Kennedy[2] state that it stains like mucus and occasionally like fibrin when appropriate stains are used. Occasionally the material filling the ducts is calcified. Sometimes desquamated epithelial cells, occasional macrophages or rare polymorphonuclear leukocytes are seen in the lumens. In the case reported by Gamble[27] the lumens were reported to contain many leukocytes.

The acini and ducts are dilated to a size that varies with the amount of secretion present and the duration of the disease. In the younger infants, large cysts are not common. Although one speaks of cystic disease of the pancreas, the cysts are essentially microscopic. The lining epithelium may vary from normal height to very low, the degree of flattening apparently depending on the amount of accumulated secretion. Hess and Saphir[33] report the finding of occasional mitotic figures in the acini. They also state that the cells were granular and the nucleus indistinct.

The accumulation of secretion in the ducts has been reported in only a few other diseases. Rich and Duff,[60] reporting on acute pancreatic necrosis, state that in 3 of their cases the disease may have been due to the presence of an abnormal secretion, since numerous ducts contained inspissated secretion that stained deeply and irregularly with eosin. In these cases the masses filled the ducts or appeared as individual masses separated by thinner secretion. Where the epithelium was desquamated, the duct wall was destroyed and infiltrated with an abundant polymorphonuclear exudate. This latter phenomenon has not been observed in fibrocystic disease and suggests that the secretion lacks trypsin, as active sterile pancreatic juice calls forth a polymorphonuclear exudate.[60]

Apparently the accumulation of secretion in fibrocystic disease results in pressure atrophy and gradual disappearance of the acinar tissue. In extreme cases all that remains are isolated, widely separated, abnormal lobules and ducts scattered throughout the pancreas, with almost complete disappearance of acini.

The islets of Langerhans are not affected as a rule, although they were thought to be decreased in several cases[30,35,51,69] and abnormal in one report.[2] Andersen[1a] states that in some cases they were seen to maintain their connection with adjacent acini or ducts but showed no other change. These histological findings are compatible with the absence of hyperglycemia and glycosuria in this disease but the finding in some cases of a high glucose tolerance curve suggests that the histopathology of the islets requires more detailed investigation.

The supporting tissue of the pancreas is, in the later stages, greatly increased and the organ appears fibrotic. It is both interlobular and intralobular (peri-acinar). There is no definite evidence of active production of collagen and fibroblasts are uncommon. According to Farber[24c] the

apparent increase is due to coalescence of the fibrous stroma following atrophy and disappearance of acinar tissue. He also states that in his series neither necrosis nor acute inflammation were seen, although in some cases, the stroma was infiltrated by large mononuclear cells and lymphocytes. Andersen[1a] makes the statement that these inflammatory cells varied from a few to a sufficient number to warrant the diagnosis of subacute or chronic pancreatitis. When the cells were numerous a few polymorphonuclear leukocytes were present as well. The cellular infiltrations were diffuse and bore no consistent relationship to the ducts. The mononuclear exudate is probably associated with products of degeneration, rather than with the presence of infection. Such infiltrations have been noted to be marked shortly after ligation of the pancreatic ducts in animals but they decrease as the pancreas becomes more and more atrophic and fibrotic.[4,29]

Search of the literature has failed to reveal any adequate explanation or description of the exact mode of disappearance of the acinar cells or the masses of inspissated secretion. The latter are presumably absorbed as no foreign body reaction has been noted, nor have the concretions been seen elsewhere than in the lumens of the ducts or acini.

Those cases showing minor lesions are perhaps of most interest and of greatest significance. As Farber[24c] states, they show no, or slight, increase of connective tissue and the large ducts are not dilated but the lumens of the small ducts and the acini contain acidophilic inspissated secretion and are dilated. In some of these earlier cases, the inspissated secretion may be present in some lobules and absent in others.[71] Furthermore, considerable numbers of normal acinar cells with acidophilic secretory granules may be seen.[1a,71]

A few cases have been reported[13,17,28,-30,32b,64,68,71] in which the pancreas is largely fatty, only remnants of ducts remaining surrounded by localized areas of fibrous tissue. The fat is of the ordinary type. This occurs mainly in the older age groups. It is presumed from the clinical history and autopsy findings that these changes are similar in origin to fibrocystic disease and are simply a far advanced stage of the disease. This finding is by no means incompatible with prolonged obstruction of pancreas, as in pancreatic lithiasis and carcinoma of the head of the pancreas[47] such may occur. Also, in experimental ligation of ducts in animals,[4] fatty infiltration has been reported. On the other hand it is also found in obese subjects without obstruction.[70] It is difficult to understand why, in a disease marked by disappearance of fat from the depots of the body, the pancreas in a few cases is extensively replaced by fat.

Respiratory System. Lesions of the lungs are almost invariably present in those patients who live beyond a few weeks. Grossly, there may be hyperexpansion of the lungs, particularly anteriorly, associated with obstructive atelectasis elsewhere. The trachea, bronchi and bronchioles are obstructed by a thick tenacious mucoid or muco-purulent exudate.[24c] The latter in some cases is abundant. There is moderate to marked bronchiectasia and bronchiolectasia, depending on the duration of the disease.

Bronchopneumonia and abscess formation are common. In most of the cases[1a] the abscesses appear to arise in relation to the small bronchi. The bronchial and bronchiolar walls are infiltrated with inflammatory cells. Blackfan and May[6] refer to these infiltrating cells as being of the mononuclear and lymphocytic type, while the lumens are filled with polymorphonuclear leukocytes. In those cases with vitamin A deficiency, keratinizing squamous metaplasia of varying degree may be present in the bronchi and trachea. Where the process is of long duration, considerable fibrosis of the lungs may occur and there may also be a chronic interstitial pneumonia.[24c] Blackfan and May[6] state that the pneumonia is primarily an interstitial one with secondary involvement of the alveoli and alveolar ducts.

The common lung invader is the staphylococcus pyogenes. Farber[24c] states that this organism appears to be of low virulence, as shown by its lack of invasiveness and by the relatively small amount of damage to the lungs, even after a process lasting for months. Farber[24c] is the only author who appears to have drawn attention to a change in the mucus glands of the respiratory tree. He remarks that the glands in the trachea and bronchi are distended and the lumens filled with a thick material similar to that found in the pancreatic tissue. Exactly what is meant when he says it is similar to that found in the pancreas is difficult to say. The material in the mucus glands of the trachea stains like mucus, while that in the pancreas does not. There does not appear to be any lamellated material as in the pancreas. He believes that the mucus in the lumen of trachea and bronchi is unusually thick and because of that he believes there has been inspissation of secretion. Undoubtedly, in many cases, the mucus glands are dilated and filled with mucus, the nuclei of the cells are flattened against the basement membrane and the cell outlines are most indistinct. However, in some cases the mucus glands appear normal.[71] Baggenstoss and Kennedy[2] state that in their cases the bronchi and their glands contained an exudate more purulent than mucoid and that only occasional glands were distended. Chronic infection of the trachea and bronchi cannot be ruled out as a cause of this histological change. Although no proof has been offered as yet that the secretion is altered, nevertheless, this is an important observation which requires further confirmation.

Liver. The liver is usually increased in size, is yellow in color and somewhat softer than normal. Andersen[1a] states that a fatty liver was found in 19 or about a third of her cases. Among these, 21% occurred in the age group of 2 weeks to 6 months and 60% in those dying after 6 months of age. Philipsborn, *et al.*[52] reported 15 out of 26 cases as showing steatosis. All their patients without fatty livers were under 9 months of age. In the few cases of Farber's[24c] series in which there was no fatty metamorphosis of the liver, the pancreas showed only slight degrees of atrophy. He believes that a rough direct correlation could be made between the amount of fat in the liver and the degree of fibrocystic disease of the pancreas. Such fatty livers are found in experimental work on dogs, where complete depancreatization even with the use of insulin results in the liver becoming fatty. On the other hand, in dogs,[31] monkeys,[11a] cats,[29] and possibly humans,[11a] where ducts have been ligated or removed with resultant atrophy of the pancreas, there has been no tendency to liver steatosis. The experimental work on the lipotropic substances (either the internal secretion of Dragstedt, or choline or lecithin) is too controversial for final evaluation. Dragstedt[21] believes it might originate from the alpha cells of the pancreas, which would be supported by the fact that in pancreatic duct obstruction, fatty livers do not tend to be found as the islets of Langerhans are intact. Cole and Howe[14] describe a case in an adult and have collected 5 from the literature in which there was atrophy of the pancreas (exact etiology uncertain), with marked steatosis of liver. These authors believe that both in the reported cases in adults and in fibrocystic disease of the pancreas in children, the fatty metamorphosis of the liver is secondary to the pancreatic disease. If this is so, one must look elsewhere than in islets for the source of lipocaic. The significance of the high incidence of fatty liver in fibrocystic disease requires investigation as to whether it is a general effect of poor nutrition or is specifically secondary to pancreatic disease.

Histologically, the hepatic cells contain large droplets of fat (fatty metamorphosis). This is uniformly distributed throughout the organ. In the earlier stages it may be most marked about the central vein. Andersen states that hemosiderin is present in the liver and Kupffer cells almost

invariably but Farber makes no mention of this, nor does any other author.

In a small number of cases (the exact incidence is unknown but Andersen[1a] reports 4 cases) there is a cirrhosis of the liver centered about the portal areas. Grossly, there are large coarse and rather irregularly distributed scars in the liver, partly marking off areas of liver parenchyma of varying size. Farber[24c] thinks the cirrhosis is due to intrahepatic biliary obstruction as he found the small bile ducts dilated and filled with eosinophilic material like that in the pancreas. He considers it likely that this secretion consists of mucoprotein and its presence, as in the pancreas, leads to obstruction and dilatation of the ducts with atrophy of the parenchyma drained by these ducts, leading to condensation of the stroma, and development of a somewhat focal type of fibrosis. It appears to be a biliary type of cirrhosis but jaundice has never been reported. Proliferation of bile ducts has been observed.[71] Initially the cirrhosis seems to be focal in distribution and even in advanced cases, normal portal areas may be seen.

Additional Findings. Grossly in 8 cases of Andersen's[1a] series the *gall bladder* was small and contained a small amount of translucent gray mucus and in 4 of these cases the cystic duct was atretic, but in most instances this organ and the bile passages are not reported upon. Andersen had 1 case with atresia of the bile duct, atrophy of the gall bladder and cysts of the cystic duct and gall bladder. Six or 30% of her own cases presented evidence of cystic duct atresia. No abnormalities of the common or hepatic ducts have been reported except in 1 case[49] which showed narrowed common and hepatic ducts. Farber mentions that frequently the gall bladder mucus glands are distended and in this respect resemble the glands of the pancreas. Furthermore, he found the gall bladder to be often small and its contents thicker and stickier than normal.

The *spleen* is normal or slightly enlarged. In 1 case with cirrhosis of the liver the spleen was large and somewhat fibrous.[35]

Changes in the *alimentary tract* are not commonly mentioned but dilatation and hypertrophy of the colon have been recorded.[1a,13] Microscopically a moderate infiltration of lymphocytes and plasma cells with occasional polymorphonuclear leukocytes and fibrosis of the submucosa of the bowel were seen in a few cases.[13,33] Two of Andersen's cases had atresia of the small bowel and 1 had an obstructing band across the ileum. All 3 died at 4 to 6 days. Farber[24c] mentions dilatation of mucus glands of the duodenum, the jejunum and the esophagus with thick material as in the pancreas. Baggenstoss and Kennedy[2] could only find dilatation of the mucus glands of the duodenum.

The *heart* is usually normal although it may show fatty infiltration. Cor pulmonale without histological changes in the pulmonary vascular tree has been noted in 1 case of fibrocystic disease with bronchiectasis.[22] The incidence of congenital heart disease is not increased in fibrocystic disease.

In those cases with severe vitamin A deficiency, keratinizing metaplasia may be found in the salivary glands, larynx, trachea, bronchi, uterine mucosa, periurethral glands, pelvic mucosa of the kidney, ureters, bladder, etc. Farber[24c] reports dilatation of the acini of the sublingual, submaxillary and parotid glands by inspissated secretion. Wilson and Dubois[72] reported a marked inflammatory infiltration of salivary glands but found only an occasional dilated acinus. Oppenheimer[48] likewise reported the finding of only a few dilated acini in the salivary glands. Inclusion bodies have been reported in salivary glands of a few cases.[9,72]

The *reproductive organs* are rarely commented on but Flax, *et al.*[26] report a case of probable meconium ileus in which the uterus and vagina were large and there was a profuse mucus exudate in the cavities of both. Sections of the uterus showed large distended mucus glands in place of the normal endometrium. Atresia of the ureters is not uncommon.[1a] Calcium salts

in the cells of the kidney tubules has been reported.[2] Sections of the skin and subcutaneous tissues show almost complete absence of fat.[27]

Meconium Ileus. Meconium ileus[10,19,20,24d,36,39,41,42,66] is a clinical term for a condition found in the newborn which is intimately associated with lesions of the pancreas identical with those found in older infants and children and called cystic fibrosis of the pancreas. It is characterized by intestinal obstruction in the newborn as a result of inspissation of the meconium which becomes so thick and sticky as to be immobilized in the gut.

This was first described by Landsteiner[42] in 1905—who ascribed it to lack of external pancreatic secretion resulting in failure of liquefaction of the meconium. This hypothesis seems to have been confirmed by Farber.[24d] The causation is either: (1) congenital stenosis or atresia of pancreatic ducts[36,41] or (2) acinar and duct obstruction in inspissated secretions[24c,24d] or (3) possible failure of normal development of the pancreas.[39]

In any case, the histological findings in the pancreas are identical with cystic fibrosis. Since at the most these patients do not live beyond a few weeks, there is as yet no evidence that they would develop the lung lesions or lesions of other secreting glands as in the older group. Therefore it is unknown whether the pancreas is the only organ involved.[24c] If Farber's hypothesis, regarding fibrocystic disease of the pancreas as being a systemic disease, is correct, one should in some of these cases expect the glandular changes to be present elsewhere in the body, but as yet there has been no note of them.

The 3 adequately reported cases of meconium ileus,[36,41,42] in which the duct of Wirsung was examined in serial microscopic sections, showed marked stenosis of the ducts, a short distance from the ampulla of Vater. The ducts, distal to the stenosis, were dilated and Hurwitt and Arnheim[36] point out that primary parenchymal fibrosis does not produce such dilatation of the distal parts of ducts. They regard these stenotic ducts as being due to a focal developmental defect and not to failure of the pancreatic ducts to recanalize in embryonic life. They suggest that the pancreatic lesion is an early intrauterine lesion, the time of origin of which might be roughly estimated by the distance the meconium has traveled. In their own case the meconium was at the ileocolic junction and in Kornblith and Otani's case at the hepatic flexure. They quote Keith[40] as stating that meconium reaches the ileocolic junction by the fourth month and the rectum by the fifth month of fetal life.

In Kaufman and Chamberlin's[39] case the ducts were not examined but in extensive sectioning of the pancreas they failed to find more than one fully developed excretory duct. No small ducts with normal tall columnar epithelium were seen. The structure of the acini was not normal and their impression was that the development of the acini was faulty and that maturation of small ducts into acini had been prematurely arrested.

Biochemical Data. Chemical analysis of the stools has always held an important rôle in the investigation of the celiac syndrome and in experimental work with animals. It would now appear, however, that their diagnostic rôle will be less important in the future and will be replaced to a large extent by estimation of the duodenal enzymes. Nevertheless, the estimation of fat, protein and carbohydrate in the stools has played an important part in the understanding of pancreatic deficiency.

It may be briefly pointed out that large amounts of the fat, protein and carbohydrate in the diet fail to be absorbed and are lost in the stool, particularly in the case of the former two. The loss of protein is particularly important and accounts, at least in part, for the failure of development and the emaciation in the subjects of this disease. The many factors that influence the loss of these substances, and the care that must be taken in their quantitative estimation and the interpreta-

tion of the latter may be found in the references in the bibliography.[1a,1d,1e,31,37,52,63]

The loss of fat, protein and carbohydrate in the stools of cases of fibrocystic disease of the pancreas is similar to that found in experimental animals in which the pancreatic ducts are ligated.[29,31] Attention is drawn to the fact that effects of ligation vary considerably from time to time in the same animal and from animal to animal,[31,37] and probably the same applies to cases of fibrocystic disease, accounting for variations in the stools and in symptoms. The processes of digestion and absorption are complicated and there are still too many unknowns, to lay down hard and fast rules. However, it may reasonably be concluded from the stool changes in fibrocystic disease and their correlation with experimental work, that the exocrine pancreatic juices are lacking.

The estimation of the duodenal enzymes has assumed great importance since the separation of fibrocystic disease of the pancreas as a disease entity. Much investigation[1c,25,44,62,73b] has been directed towards this problem and as the result of advances in technique, clinical interest has been regained in the quantitative estimations of the enzymes. Their estimation in fibrocystic disease is essential for clinical diagnosis.

In fibrocystic disease the values are very low or zero for all enzymes, well below the low limits of normal cases or of other diseases. Estimation of trypsin only is necessary. The low values persist even on repeated examinations.[25] It is important to note that the duodenal fluid is scanty and very viscous as might be expected from the histological appearance of the secretion in the pancreas. Intravenous secretin fails to affect the volume of the fluid or its enzymatic content,[44,53] except possibly in early cases.

The results of enzyme estimations confirm the histological appearance of marked pancreatic dysfunction. It is not known whether the pancreas fails to produce enzymes or whether the escape of these is prevented by increased viscosity of the secretions. As some cases have a small amount of enzymes present, it may be that enzymatic secretion is normal but the secretions cannot escape. On the other hand their presence might be accounted for by areas of normally functioning tissue, such as are sometimes seen histologically. Much more correlation between enzyme activity and histological findings is required before any conclusion can be reached.

As regards gastric analysis little or no information can be found in the literature but Andersen (personal communication)[1f] states that in general, except in extreme marasmus, the acidity and rennin content of the gastric juices are normal. This is a rather important observation from the viewpoint of Farber's theory that this disease may be a general one affecting secreting glands. The stomach would then have to be excluded from the general thesis.

In general, chemical examination of the blood is not of great value. The glucose tolerance curve may be flat[1b] or high.[32b] Blood cholesterol tends to be low.[1a,1b] Total lipids and their fractions are essentially normal.[43] The fasting vitamin A content of the serum is low[52] and the vitamin A absorption curve is flat.[6] These findings seem to confirm the failure of adequate absorption, particularly of fat, but throw no light on the etiology of the disease.

Etiology and Pathogenesis. Before commencing to discuss these aspects of the disease, it is well to remember that the pancreas is but one supply depot of enzymes in the complicated and lengthy process of digestion, commencing in the mouth and ending with the expulsion of the undigested residue. In addition to the digestion of food, there is the equally complex process of absorption. One or both mechanisms may be affected and to distinguish the rôle of each in disease, is difficult. Experimental work has defined areas in the gut where certain processes are known mainly to take place but their

correlation with other parts and their ability to take over functions, one from another, is not clearly defined. For years arguments have been going on as to the importance and necessity of the exocrine secretions of the pancreas in digestion. So far as dogs, rabbits and cats are concerned, it is apparent that the exclusion of the pancreatic juices does affect their health, although not in a sharply defined manner. Much care must be taken to exclude all pancreatic juices from the intestine, for functional disturbances can be prevented if only 1 cm. of pancreas is left attached to a patent duct.[56]

The importance of the pancreatic enzymes in digestion has recently been again clouded by the results of surgical exclusion of the pancreatic juices in humans.[11a] A majority of the cases in a small series did not develop symptoms of pancreatic dysfunction. Brunschwig,[11a,11b] after transecting the head of the pancreas and ligating the ducts in monkeys, has found that they live and thrive for several years without evidence of disturbed digestion. There was no evidence at autopsy that the pancreas was functioning.

As a result, in any disease which shows marked pancreatic change, one must be careful in attributing a host of symptoms, signs and biochemical findings entirely to dysfunction of this organ. Therefore, in fibrocystic disease of the pancreas, although many of the celiac symptoms are attributable to deficiency of the pancreatic secretion, one must be wary of assuming that this disease entity is dependent wholly on the pancreas. Such an assumption may be later proven to have been wrong. In short, the pancreas may be only the symbol of a much more generalized disease, as pointed out by Farber.[24c]

Nevertheless, all discussions of etiology and pathogenesis center on attempts to explain the changes in the pancreas. There is general unanimity in regarding the pancreatic lesions as of obstructive origin,[1a,24c] but the exact mechanism of obstruction is uncertain. The very nature of the lesion with its dilated acini and ducts filled with secretion and the ultimate atrophy and replacement fibrosis, indicates obstruction. The end results are similar to those following ligation of the ducts experimentally and in cases of obstructed ducts.[4,47] Other than the final result, it is questionable whether the changes following sudden complete obstructions of the pancreatic ducts are comparable to the obstruction in fibrocystic disease. In the latter disease the obstruction is probably partial at first and slowly progressive, possibly never to become complete.

The etiological theories, regarding the mechanism of obstruction, can be discussed under three headings: (1) Infection, (2) Vitamin A deficiency, (3) Congenital defect: (*a*) Obstruction from atresia, (*b*) Obstruction from production of an abnormal secretion.

Infection. It has been suggested[1a] that the presence of minor degrees of inflammation in the pancreas might indicate a fetal or post-natal infection. The main evidence against infection is the absence of a known infecting agent; the failure to find inflammation of the pancreas in the presence of gastro-intestinal infections; the absence of chronic biliary infection in children and the presence of similar inflammatory infiltrations in the degenerating pancreas in experimental ligation of the ducts. The familial incidence is also against fetal inflammation. Congenital lues is ruled out by the failure to find evidence of syphilis in the majority of cases. Although a few cases show inclusion bodies in some of the tissues,[9] the incidence is no higher than that found in routine autopsies.[24c] Finally, inflammation of the pancreas does not explain the high incidence of lung lesions in this disease.

Vitamin A Deficiency. The chief difficulty in assigning the causation of fibrocystic disease to this deficiency is the absence of squamous metaplasia of the pancreatic ducts of such extent as to cause obstruction, except in rare cases. Extreme cases of vitamin A deficiency may show no pancreatic change.[7] There

is no evidence that pre-natal deficiency of vitamin A is a causative factor.[1a]

The balance of evidence suggests that vitamin A deficiency is a complication and not the cause of the disease. However, this deficiency, with considerable reason, has been regarded as the cause of the lung lesions.[1a,49] Here again, although the relationship has not been clarified, the weight of evidence appears to be against vitamin A deficiency as a primary cause of the pulmonary infection.[24c] Lung infection is not common in idiopathic celiac disease in which vitamin A absorption is poor, or in adult humans or animals with pancreatic duct obstruction. On the other hand, Oppenheimer's patient with straightforward duct atresia died with pulmonary disease, which may have been secondary to the vitamin A deficiency present. Until direct evidence is found, the relationship of the pulmonary lesions to vitamin A deficiency will remain obscure.

Congenital Defect. The fact that the lesions are frequently present at birth or develop in early infancy, as well as the familial incidence and the frequent presence of atretic ducts elsewhere, suggest strongly that the basic abnormality is a congenital one. Yet, it differs from most congenital anomalies, such as atresia of the bile ducts or congenital heart disease, in the absence in most cases of a gross anatomical defect. The variation in the severity of the microscopic lesions and the survival of a considerable number of patients well on into childhood is puzzling and one must assume that the basic defect continues to operate with varying intensity long after birth and in a different manner from a clear-cut anatomical defect. For these reasons, the reviewer believes it important in fibrocystic disease to separate those cases secondary to congenital atresia of the ducts from those without atresia. There seems to be no reason why pancreatic duct atresia could not occur as an isolated anatomical defect, in which case the secreting glandular structures of the bronchi and other epithelial tissues would not necessarily be congenitally abnormal. On the other hand, in those cases without atresia, it would be reasonable to hypothesize, as Farber has done,[24c] that there is a generalized defect of the exocrine glandular secreting tissues, including the pancreas, if the anatomical evidence supported the theory.

(*a*) *Congenital Duct Atresia.* There seems to be no doubt whatsoever, that a few cases of fibrocystic disease of the pancreas, excluding cases of meconium ileus, have their origin in atresia of the pancreatic ducts.[5,48] Although Andersen[1a] and Farber[24c] state that congenital atresia of the main ducts is uncommon (except in meconium ileus), this is by no means certain as most of the examinations of the ducts appear to have been made by gross dissection and in but few cases have microscopic serial sections been made. The difficulty of tracing these ducts grossly[1a] in infants emphasizes the necessity of careful serial microscopic examination of these ducts. Furthermore, the head of the pancreas and second part of the duodenum should be removed *in toto*, routinely at autopsy, and kept until sections of the pancreas are examined. Andersen has suggested[1a] that when stenosis or atresia is present it may have been the result rather than the cause of the fibrosis.

A theory which comes under the heading of atresia is that proposed by Rauch, *et al.*[59] This is based on the common origin of the anlagen of the lung and pancreas from the entodermal tube. In order to bring the pancreatic and lung lesions into relationship, they proposed that at some stage of development of these organs, as a result of budding, a constriction of some of the excretory ducts of both organs might occur, giving rise to congenital bronchiectasis and congenital fibrocystic disease. Although some pancreases show apparent failure of proper development,[39] no factual proof for this theory has been adduced.

Another form of obstructive lesion described by Baló and Ballon[3] (not necessarily congenital), consisting of focal epi-

thelial metaplasia of the ducts of the pancreas, causing small duct obstruction, is ruled out by their absence in the 5 cases examined by Rich and Duff.[60]

(*b*) *Theory of Abnormal Secretion.* This reasonable, intriguing and all embracing hypothesis has been developed by Farber[24c] in an attempt to explain particularly the association of lung and pancreatic disease and to account for the pancreatic lesions in the absence of main duct atresia. In brief, he believes it is a systemic disease, of which the pancreatic changes are only a part, although they may predominate. It is based on a statement by Blackfan and Wolbach[7] that the pathogenesis of fibrocystic disease of the pancreas lay in the production of an abnormal secretion, but Farber has extended the theory to include other epithelial lined organs. His study of 87 advanced cases and of about 350 early cases[24b] leads him to believe that the basic lesion is a physically altered secretion causing obstruction of acini and small ducts, later involving large ducts and leading to atrophy and fibrosis. The definite absence of duct atresia which has been proven in a few cases,[1a,2,49,64] the absence of any large duct dilatation in some cases[24c] and the thick scanty duodenal juices, is evidence in favor of an abnormal pancreatic secretion. Furthermore, the presence of aberrant pancreatic tissue showing similar changes to the pancreas, in 3 cases,[1a,24c,68] argues that intrinsic biochemical alterations of the secretion are fundamental. Unfortunately there is no direct proof of this alteration nor evidence as to its nature.

The evidence for altered secretion in other epithelial lined organs is less convincing. Farber[24b,24c] claims that many cases show dilatation of glands and inspissation of secretion in the trachea, bronchi; esophagus, duodenum, gall bladder, salivary glands and jejunum. All cases reported previous to his publication make almost no mention of such findings, although in 2 cases[48,72] the salivary glands showed minimal dilatation of acini. As has been previously indicated, the one study reported[2] since then was not strikingly confirmatory. It is apparent that confirmation of the extra-pancreatic lesions is still lacking, although it may be noted that the liver lesions of the associated cirrhosis bear a striking resemblance to those of the pancreas.[71]

As further proof of his theory, Farber[24c] has reported a case in which the lungs and liver showed the above-mentioned changes in a patient who had no symptoms referable to pancreatic achylia and in whom at autopsy a normal pancreas was found.

Farber has suggested[24c] that the character of the duodenal drainage and the inspissated secretions in the acini are reminiscent of vagal stimulation (as has been known for years vagus stimulation produces a thick secretion, high in enzyme content). He suggests that a disturbance of parasympathetic innervation or an autonomic imbalance in nervous control of secretions in the pancreas and in mucus glands may be at fault. He has produced[24a] a similar lesion in the pancreas of kittens by the injection of a parasympathomimetic drug (pilocarpine) over a period of 2 to 8 weeks. Extreme loss of weight and severe nutritional disturbances were the chief clinical features. As far as the reviewer can find, no one has repeated these most interesting experiments.

Summary. Fibrocystic disease of the pancreas is the name given to a disease, whose etiology is completely unknown, because the pancreas shows the most striking histological changes. In the absence of a better name, one must remember not to place too much emphasis on the pancreatic changes until it is proven to be essentially a disease of the pancreas alone, or otherwise. In view of the contradictory experimental findings in animals and humans where exocrine secretion of the pancreas has been excluded from the gut, one must proceed most cautiously in assigning to the pancreatic lesions in this disease an all-important rôle.

Clinically, the disease is characterized by severe respiratory infection and gastro-

intestinal symptoms and signs of the celiac syndrome, the former tending to predominate under 1 year of age and the latter over 1 year of age. In the neonatal period it gives rise to the clinical entity called meconium ileus. The disease appears to be congenital and not infrequently familial.

Pathologically, the essential lesion in the pancreas is obstruction and dilatation of acini and ducts by an inspissated secretion with secondary atrophy and fibrosis. Associated with this is the almost constant finding (except in very young infants) of chronic respiratory infection with a mild bronchiectasis, abscess formation in the lungs and bronchopneumonia. The liver shows marked fatty metamorphosis in about a third of the cases and in a small percentage biliary cirrhosis of a somewhat focal nature is present. In meconium ileus the pancreatic lesions are present and the bowel is obstructed by a thick mucilaginous meconium which is formed in the absence of pancreatic juices.

Several etiological factors have been put forward, of which only two can reasonably be considered in the light of our present knowledge. The first is the theory of congenital atresia which postulates that there is a congenital atresia or stenosis of the pancreatic ducts, or failure of normal development of the pancreas. Atresia is more common in meconium ileus and unquestionably accounts for a certain number of cases in older children. With more detailed and adequate examination of the duct system, a definite number of cases will undoubtedly be isolated from the group and placed in their proper etiological relationship. However, a larger proportion of cases appear (and one uses this word advisedly) to be due to an intrinsic obstruction, probably commencing in the acinar area which may be due to an unknown congenital defect in the function of the pancreatic acinar cells themselves, resulting in an unusually thick secretion. This theory, developed by Farber, is most important but lacks adequate proof. He believes, in addition, that there is a widespread involvement of variable degree, of other mucus secreting structures in the body as shown by the change in the physical character of the mucus produced. There may be either a deficiency or insufficiency of mucinase required for the maintenance of secretions in the normal state. The type of secretion present is reminiscent of the results of vagal stimulation. Neither Farber's histological findings nor his experimental work have as yet been confirmed.

The possibilities of further studies on fibrocystic disease of the pancreas are almost unlimited. Detailed and careful pathologic studies alone would be useful in clarifying many obscure points, while there is a wide scope for experimental work in the pathologic physiology. Finally, it may be stated that in spite of the spate of literature in the last 8 years, the great progress in diagnosis, and the slight progress in treatment of this disease, the etiology is not known and in particular the relationship of the pancreatic and lung lesions has not been elucidated.

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PREVENTIVE MEDICINE AND EPIDEMIOLOGY

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MATERNAL DISEASE AS A PRINCIPLE IN THE EPIDEMIOLOGY OF CONGENITAL ANOMALIES

WITH A REVIEW OF RUBELLA

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Congenital Anomalies Following Rubella. In the early months of 1941,* an unusual number of cases of congenital cataract were observed in Sydney. When they continued to appear, in numbers indicative of no chance phenomenon, seri-

* In 1940 the Medical Journal of Edinburgh carried an editorial, not to call attention to anything new about rubella, but only to celebrate the 100th anniversary of the appearance in "The Journal" of the first description of the disease "in our language."[12] The editorial opens, "German measles has been in the news of late, and we hear of medical officers of health, headmasters, headmistresses, and others clamoring to have it deprived of . . . its place as a notifiable disease and relegated to the limbo of minor exanthemata . . . a nuisance rather than an illness." The identity of "Rötheln" had already been established in the German literature and Dr. Robert Patterson of the City of Leith, being convinced from many cases "which lately prevailed in this neighborhood that it possesses characters peculiarly its own," was induced to present a comparison with measles and scarlet fever in tabular form, and the clinical aspects of the disease quaintly captured in his phrase that "the little patients are observed to draw nearer the fire." The editorial concludes that "little seems to have been added in the intervening hundred years," a statement borne out by reference to almost any text written up to within very recent years where the disease is quickly dismissed as of no consequence in itself and without complications or sequelæ.

If "little seems to have been added in the intervening hundred years," the editorial commemorating the centenary of Patterson's description of the disease set the stage, as it were, for events to come within the next few months, in the first year of the second century, which dramatically removed rubella from the "limbo of minor exanthemata." Its role as a "nuisance rather than an illness" was unmasked by a group of Australian investigators whose clinical acumen and epidemiological insight no doubt would be applauded by Patterson himself, and will be celebrated in 2041 by another editorial in the illustrious Journal.

Rubella now takes its place as one of that group of diseases where infection in the many is trivial, but where sequelæ in the few constitute major problems of epidemiology and preventive medicine. As if to outdo its fellows, and to elude medical science, rubella is so mild and its sequelæ so severe that any connection between them would hardly be suspected. To cover its tracks, unlike poliomyelitis with its paralysis on the third day and the mastoid or pneumonia on the heels of the rash in measles, it put so much distance between itself and its sequel—at least several months, and into the next generation—that it was not detected until with epidemiologic overconfidence from a long period of quietly "getting away with it," so to speak, it tried to make up for lost time with its "virgin soil" outbreak in Australia. This circumstance related to us by Burnet on his visit to this country in 1944,[6] is summarized by Scholes as follows: "During the past 35 years the first large epidemic of rubella occurred in 1914, the next in 1923 and the last in 1937. The 1937 outbreak spread to all states, but had not died out in remote districts when war broke out in 1939. In that year, with the mass movements of young people from state to state and from country districts, the disease became epidemic, sweeping through the whole country and waves had recurred until fairly recently. Between 1923 and 1937 there was no opportunity for anyone to contract rubella and from 1937 to 1942 there was every opportunity, the vast majority of young adults and women of child-bearing age being susceptible."[35]

Finally, as if to shield itself from medical intrusion, rubella selected as its channel of operation a condition least likely to be interfered with in the interest of prevention, for pregnancy is so dominated by religious, moral, emotional and legal considerations, that its purely medical aspects may be secondary to them. Indeed at times they have been taken into consideration only insofar as they afforded a basis for some action dictated by these other attitudes. The Pilgrims, for example, whose major epidemiologic pursuit seems to have been the search for sin, and who believed the period of gestation was 9 months to the day, sent many a young couple to the stocks "for having a childe before the natural time of woman after marriage."

ous thought was given to their causation.[19a,19b,19c] Their almost epidemic occurrence, their ophthalmologic characteristics and their association with congenital heart disease suggested to Gregg that the etiology was a constitutional, toxic, or infectious disturbance in the fetus, rather than a purely developmental defect. Calculations relative to the stage of pregnancy, when the congenital anomalies must have originated, showed that it coincided with the period of maximum intensity of a widespread epidemic of rubella. Gregg collected 78 instances of congenital defects, the basis for which was found to lie in maternal rubella during pregnancy. His reports were confirmed and the investigation extended by Swan and co-workers.[41a,41b]

TYPES OF CONGENITAL ANOMALIES. The special ophthalmologic features were cataract, usually bilateral, and of a subtotal type associated with nystagmus and a sluggish pupillary reaction to light; in some cases, an atrophic appearance of the iris; and rarely microphthalmia and buphthalmia.

On histologic examination, Swan[40] found the peripheral lens fibers showed evidence of degeneration or necrosis, and were separated by vacuoles, while the central core was amorphous in appearance.

The cardiac manifestations were a harsh systolic murmur over the precordium, usually loudest over the base of the heart or pulmonic area, and absence of cyanosis. In each of 3 afflicted infants autopsied by Swan,[40] the ductus arteriosus was widely patent, there was variable patency of the foramen ovale, and 1 baby had an interventricular septal defect. As regards malformations of the heart Swan quotes Bedford and Brown[3] who state that "the critical period for the development of congenital cardiac defects is from the fifth to the eighth week of intrauterine life, during which the septa are forming, the bulbus cordis is undergoing involution, and torsion of the great vessels is taking place." Subsequently, other forms of congenital heart disease accompanied by cyanosis have been reported.

Green and Dogramaci[18] who reviewed the records of 434 children studied at the Children's Hospital, Boston, for congenital heart disease, found a history of maternal rubella in 4 instances and exposure of a susceptible mother in 1 instance. They presented data as shown in Table 1.

Assuming that there had been 100% accuracy in diagnosis and reporting of the disease (in the hospital records), the inference would be that rubella was the etiologic agent in approximately 1% of all types of congenital heart disease. It is reasonable to conclude that reliance on hospital records alone would result in the loss of many cases in which the infection had been regarded as trivial, forgotten or merely overlooked, and that the true figure will eventually be shown to be substantially higher. That this will prove to be the case, is also suggested by the work of Conte and co-workers[8] who surveyed the records of all congenital anomalies at the Vanderbilt University Hospital. Of 120 anomalies there were 4 cases of congenital heart disease in which maternal

TABLE 1.—DATA IN 5 CASES OF CONGENITAL HEART DISEASE RELATED TO RUBELLA (GREEN AND DOGRAMACI[18])

Stage of pregnancy when rubella occurred	Presence of cataracts	Nature of cardiac defect	Physical signs	Roentgenologica findings
1 month	+	Tetralogy of Fallot	Typical, cyanosis	
3 months	+	Uncertain	Rough systolic murmur over precordium	"Globular heart"
2½ months	+	I.V. septal defect	Harsh to and fro murmurs in pulmonic area, with grade 2 murmur over apex	"Globular heart with broad base"
1 month	0	Patent ductus arteriosus	"Typical"	Typical
2 months*	0	Pulmonary stenosis; I.V. septal defect	Cyanosis	

* Exposure to rubella.

rubella appeared to be the inciting agent. The incidence in this series would be around 4%, since other anomalies as well as heart disease are included. The higher figure is to be attributed to additional information obtained by a questionnaire mailed direct to all mothers.

In addition to the malformations enumerated above, deaf-mutism has been reported by Swan and his co-workers[41a,41b] in a total of 12 out of 43 children born to mothers who suffered from rubella during pregnancy. Some form of deafness was noted by Carruthers[6] in 74 out of 102 such cases. Muteness when present was regarded as secondary to deafness. In an autopsied case, the outstanding defect was the total absence in both ears of any differentiation of the primitive cells to form the organ of Corti.[40]

Evans[15] found that of 34 babies whose mothers suffered during pregnancy from rubella, 23 exhibited congenital dental abnormalities, in 18 cases major in nature, such as "sharklike" and "pointed" incisors and enamel hypoplasias. All except 2 of the infants showed other congenital malformations.

Finally, it was noted that most of the babies were of small size, ill-nourished, and difficult to feed. No less than 15 of the 78 patients studied by the Australian workers were dead by the end of 1943, and many of those living showed mental as well as physical retardation.

These reports have been confirmed in this country by Reese,[36] Rones,[37] Erickson,[14] and Albaugh,[1] who add 18 more cases to the literature.

Stage of Pregnancy. The combined work of the authors cited indicates that the average period of the pregnancy when rubella occurred was as follows:

For cataract	6 weeks
For deafness	9 weeks
For cardiac anomalies	5–10 weeks
For deformed teeth	6–9 weeks

Carruthers, however, felt that although the average date was of some significance, "the importance of the stage of pregnancy at which infection occurs is that, if it is in the first 6 weeks, fetal damage may be widespread and include the eyes, both divisions of the ears, the heart, and perhaps many other parts. After the sixth week, the eyes may escape, the heart may be spared, and the semi-circular canals may become normally developed; but the cochlea is still likely to be damaged." No correlation could be established between the clinical intensity of the maternal infection and residual fetal damage. There would be no reason for doubting that the disease in question was rubella, or that the syndromes produced were new, except as they were newly recognized. Chandler[7] is of the opinion, however, that the resulting type of congenital cataract is seen more frequently now than formerly. It is not known to what extent this increase may be accounted for by the better practice of obstetrics and pediatrics which now bring to term more pregnancies which result in a living baby. This seems to be the case in retrolental fibroplasia, described by Terry.[43]

Frequency of Anomalies. Most of the earlier studies have started with cases of congenital defects of the sort described. The finding of maternal rubella in a high percentage of the cases has been interpreted by some as indicating that the defects always follow the infection. For example, it has been stated that "available data would suggest that 100% of the mothers who contract rubella in the first 2 months, and approximately 50% of those who contract it during the third month, will give birth to infants with congenital anomalies."[1] As pointed out by Albaugh, many questions have been raised which will require for their answers extensive epidemiologic studies. An editorial in the *Lancet* in February, 1946,[13] states that "It is four years since Gregg in Sydney reported that congenital malformations . . . were occurring in infants whose mothers had had German measles early in pregnancy . . . yet nobody seems to have taken the few simple steps needed to find out whether rubella is a serious cause of congenital

defects . . ." The only study which represents an adequate attempt to determine the numerical probability of congenital anomalies following rubella during pregnancy is that of Fox and Bortin.[17] They found that of 152 married women who contracted rubella during 1942, 1943 and 1944, 11 had rubella during pregnancy: 5 during the first 2 months, 4 during the second to the fourth month, 1 in the seventh, and 1 in the ninth month. They found only 1 of 11 cases (rubella in the first month of pregnancy) which "evidenced a pathologic course." The child in this case was stillborn. However, they present a second instance (rubella in the second month of pregnancy) in which the outcome was a "blue baby, sinus disease, otitis media, hydrocephalus (which receded spontaneously), perfectly normal at present."

Approaching the problem by the same method, we have investigated 1300 cases reported to Boards of Health of 2 Massachusetts communities for instances of rubella in pregnancy, and have found 4 as follows:

Case	Length of pregnancy before rubella	Result
1	2d month	normal baby
2	2d month	mental retardation
3	4th month	normal baby
4	9th month (12 days antepartem)	normal baby

If these 4 cases are added to the 11 cases reported by Fox and Bortin, there are 15 instances of rubella in pregnancy where data on the outcome are available. The disease occurred in the first 3 months of pregnancy in 11, with 8 normal and 3 abnormal babies resulting: 1 infant with spontaneously subsiding hydrocephalus, 1 mentally retarded child, 1 stillborn infant, and 8 normal infants. Four cases occurring from the fourth to ninth month all resulted in normal babies.

In this small series, the morbidity in the baby is 27%, when the disease occurred in the first 3 months of pregnancy.

That a study based on cases reported to Health Departments may represent an incomplete picture of this phase of the problem, may be seen from the following cases found in the course of this study, in only one of which had the rubella in the mother been reported.

To these 5 can be added 2 of our cases in which spontaneous abortion resulted.

Thus, on the basis of admittedly incomplete data, it would appear that the risk of anomalies in the infant may be upwards of 25% following rubella during the first 3 months of pregnancy. Whatever the final incidence turns out to be, it will be sufficiently high as to constitute a problem of a very serious nature to the parents concerned and their obstetrician. Available data do indicate that the first 3 months of gestation is by far the most vulnerable period. Of 100 cases reported by Carruthers, Swan, Erickson, Reese, Rones, Green, Albaugh and Kraus,[29] 94% of the mothers were recorded as having suffered the infection during the first 3 months (Table 3).

Abortions and Stillbirths. Nowhere in the literature, except for Fox's mention of a stillborn baby following maternal rubella, have we found mention of the possibility that death of the fetus and spontaneous abortion may result. That this may occur is suggested by 4 cases of which we have personal knowledge (Table 4).

Congenital Anomalies and Other Maternal Diseases. No studies have appeared with respect to congenital defects following other maternal infections, but 3 instances of such defects, 1 following chicken pox, 1 poliomyelitis, and 1 influenza, have recently been observed in Massachusetts.

Case	Length of pregnancy before rubella	Defect	Reported to Board of Health
1	2 months	Deafness	No
2	2 months	Mental retard	No
3	2 months	Heart disease, cleft palate	No
4	1 month	Heart disease	Yes
5	2 months	Cataract, heart disease, deafness	No

Mann[32] has expressed the opinion that anomalies may follow other infections, and Swan[40] has postulated that more severe virus infections, such as rubeola, may kill the fetus, whereas the milder rubella merely damages the surviving fetus. Lynch[31] has called attention to the high mortality of the fetus in maternal measles and smallpox.

The established relationship between maternal rubella and congenital anomalies, together with these observations of anomalies following other maternal illnesses, raises the question to what extent maternal disease in general may represent a principle in the etiology of congenital anomalies—heredity, for example, being another.

POLIOMYELITIS. In the course of a study of poliomyelitis in pregnancy from other points of view[26b] records have been collected concerning 264 cases. Of these, some statement concerning the infant is given in 131. In Table 2 the outcome of the pregnancy is shown according to the month of pregnancy during which the mother contracted poliomyelitis. Cases where the mother died of the disease with the fetus *in utero* are not included. Pregnancy terminated or resulted in a dead fetus in the 2 cases in the first month, in the majority of cases in the second month, and in 5 of the 10 cases in the third month. Thereafter the outcome was, as a rule, a normal child if the report "normal delivery" can be so interpreted. In 3 instances (second, third and seventh months) there were defects in the infants while in 3 others (ninth month) the infant was reported to have suffered from poliomyelitis. The evidence suggests that the risk to the fetus in maternal poliomyelitis is, as in German measles, high if the disease occurs in the first 3 months of pregnancy and less in later months. It is not clear, however, to what extent the hazard to the fetus may be the result of paralysis in the mother.

MONGOLISM. Ingalls and Davies[27] are

TABLE 2.—CONGENITAL DEFECTS FOLLOWING MATERNAL RUBELLA BY MONTH OF PREGNANCY

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Carruthers[6]	2	8	6	3		1			
Swan[41a]	12	19	10	2					
Erickson[14]	7	4							
Reese[36]	3								
Rones[37]	1	1	2						
Green and Dogramaci[18]	2	2	1						
Albaugh[1]	2	4	3						
Kraus	1	4							
	30	42	22	5		1			

TABLE 3.—POLIOMYELITIS IN PREGNANCY: OUTCOME WITH REFERENCE TO INFANT BY MONTH OF PREGNANCY

Month of pregnancy	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	
Total cases	*2*	*9*	*16*	*12*	*16*	*19*	*13*	*13*	*31*	*131*
Normal child		1	6	4	7	8	5	8	15	
"Normal delivery"		1	4	6	3	6	2	2	11	
Cæsarian section					1		1	1		
Total	0	2	10	10	11	14	8	11	26	92
Stillborn or died shortly	1	1	3	2	5	5	4	2	2	
Therapeutic abortion		1								
Abortion	1	4	1							
Miscarriage			1							
Total	2	6	5	2	5	5	4	2	2	33
Polio in baby									3	
"Lame"							1			
Congenital defects*		1	1							
Total		1	1				1		3	6

* 1 bilateral clubfoot, "not polio;" 1 congenital heart.

collecting clinical data on prenatal disease in relation to mongolism. A preliminary survey reveals the following 5 instances of intercurrent disease among 50 mothers who subsequently gave birth to a mongolian idiot.

Intercurrent disease	Stage of pregnancy
"Flu"	2 months
Rubella	2 months
Mumps*	2 months
Mastoidectomy	2 months
Pleurisy—in bed 3 weeks	"in 3d month"

* Patient of Dr. Richard Tefft.

In addition, in this survey they noted the following possibly relevant findings: 3 mothers were respectively noted in hospital records as being "sick throughout entire 9 months," "continually vomiting," and "sick all 9 months, nausea and vomiting, pain in the right side—hemorrhaged before delivery." A fourth had "grippe" during the sixth month, a fifth an "afebrile rash, last trimester," and a sixth was an undoubted alcoholic. A seventh mother had a cholecystectomy between a second pregnancy (normal) and a third (stillborn), followed by a fourth pregnancy (mongolian). One mother with 8 normal children had a ninth (miscarriage at 3 months), followed by a tenth pregnancy (mongolian). In this case there was "pink staining" at the time of period, second month." Of 50 mongols then, there were 5 instances when a specific, relatively incisive maternal illness was present at the eighth week of gestation, and 5 others in which a more chronic maternal disorder included the eighth week of fetal life.

The number of cases in which there was intercurrent disease, may not be significant in itself, but the tendency to localization of the particular maternal disease at a specific stage of pregnancy is provocative. In addition, this finding is even more interesting in consideration of associated congenital anomalies other than primary mongolian stigmata (which included 12 instances of deformed little fingers) as follows: imperforate anus, 3 cases; webbing of the fingers, webbing of the toes, and cleft palate, 1 instance of each. The correlation of these deformities with the formation of these particular structures at about the eighth week of fetal life is noteworthy. Ladd and Gross state that "membranous imperforate anus results from a persistence of the anal membrane, an arrest at about the eighth week."[30]

TABLE 4.—RUBELLA IN RELATION TO 4 CASES OF ABORTION AND STILLBIRTH

Case	Length of pregnancy before rubella	Estimated death of the fetus	Hemorrhage before delivery	Delivery of dead fetus
1	8 weeks	6 months	2 weeks	8 months
2	16 weeks	5¾ months	1 week	6 months
3	Uncertain	Uncertain	None	9 months
4	4 weeks	Immediate	None	6 weeks

According to Gray "the limbs begin to make their appearance in the fourth week as small elevations or buds at the side of the trunk. The axial part of the mesoderm of the limb-bend becomes condensed and converted into its cartilagenous skeleton. By the sixth week the three chief divisions of the limbs are marked off by furrows—the upper into arm, forearm, and hand; the lower into thigh, leg, and foot." In the phalanges ossification begins in the body about the eighth week. And again, "the deformity known as cleft palate results from a non-union of the palatal process." "The union . . . commences in front, the premaxillary and palatal processes joining in the eighth week, while the region of the future hard palate is completed by the ninth."

Ingalls[26] is making a further analysis of mongolism from the point of view of its embryologic development.

Rubella—General Considerations. Rubella now takes on the status of a disease worthy of the best in diagnosis, reporting, treatment, prevention and research. With this in mind, a reconsideration of the disease is presented.

CLINICAL MANIFESTATIONS. Rubella is a mild, infectious, self-limited disease, ostensibly of the upper respiratory tract, though as is now obvious, of a constitutional nature. Its clinical importance lies in its rare complications. These include secondary infections, encephalitis, neuritis, and now the fetal disturbances already mentioned, and even death.

The infectious agent is a filterable virus, and filtered nasopharyngeal washings give rise to the infection in susceptible human beings upon subcutaneous injection.[22] The virus particles are demonstrable by fluorescent microscopy in nasal and pharyngeal washings, in blood and in blister fluid of infected patients.[39] Habel[20] has inoculated macacus rhesus monkeys subcutaneously, intraperitoneally, intranasally, and intravenously, with the subsequent development of a mild infection characterized by a leukopenia, relative lymphocytosis and fever (average .5° C.), followed by a "very light scattered macular rash on the face, abdomen and thighs, starting about the eleventh day, and followed by desquamation." He was also successful in culturing the virus on the chorio-allantoic membrane of the chick embryo in 2 of 4 attempts.

The natural incubation period for man is generally considered "14 to 21 days; usually about 16 days."[9] In an outbreak of rubella in a boys' boarding school[42] 100 of the 106 cases in the first wave followed the initial case by 15 to 23 days (*i. e.*, 18 ± 3 days from rash to rash). Peak incidence occurred at 18 days, and there were just 18 days between the theoretical peak of this primary wave, and the peak incidence of a subsequent secondary wave (Chart 1). Thus the interval between cases when it can be established is sufficiently distinct from that of measles, for example, to be used in differential diagnosis.

The prodromal stage is ordinarily 12 to 24 hours though it may be prolonged beyond this period and is often characterized by the development of mild "grippy" malaise, coryza and conjunctivitis, together with enlargement of the post-auricular nodes. In a series of 305 cases, Humphrey[24] found that "the most constant symptom was post-auricular adenopathy."

However, it must be stressed that constitutional symptoms may be slight and in school epidemics it has been observed that many infected boys may not even bother to come to sick call.

Most definitions of rubella define the condition as a febrile disease and Humphrey reported in his series that "the temperature ranged from 99° to 100° F. in the majority of cases, and remained elevated only 1 or 2 days. In a few instances it rose to 101° and in still fewer to 102° or even 103°." In Chart 2 the temperature curves of 20 adolescent boys

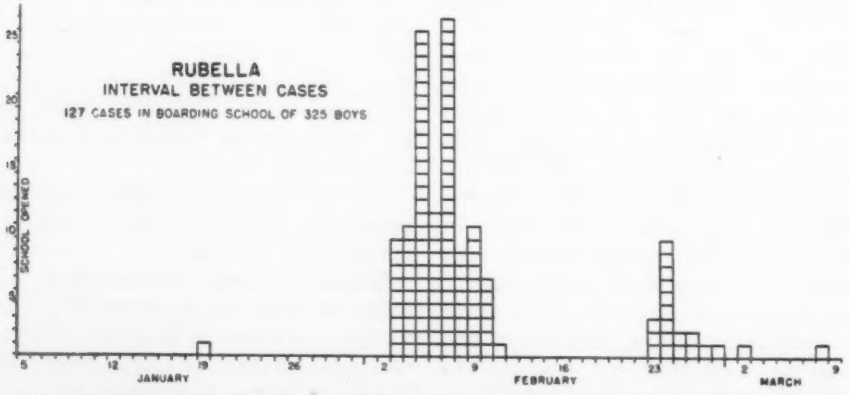

CHART 1.—Boarding school outbreak of rubella, indicating interval between cases averaging 18 days as measured from rash to rash.

selected at random are shown. They are superimposed in groups of 5, and roughly grouped according to whether the temperatures are subnormal, normal, elevated but with a quick return toward normal, and elevated with a slower return toward normal. There appears to be no characteristic curve and many curves are afebrile, or even subnormal throughout the entire course of the disease.

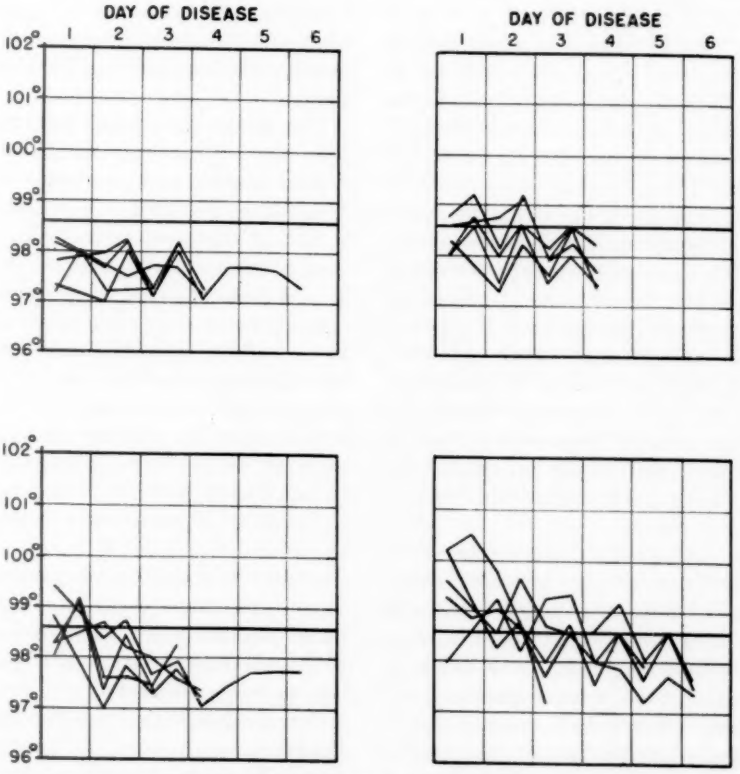

CHART 2.—Temperature curves observed in 20 cases of rubella arranged roughly according to type.

The mucous membranes of the nasopharynx are typically injected, though there is no true enanthem. The bulbar conjunctivæ are suffused so that the white of the eye is more generally pink than inflamed as in measles. The exanthem developing at the end of the prodromal stage is ordinarily manifested as a fine rose-pink maculopapular "pin point" rash, usually beginning on the face and becoming prominent over the trunk, abdomen, and extremities. Occasionally the macules will be as large as a pea, rose-red or darker, and may coalesce.

If the characteristics of the disease cited above appear to give the impression that rubella is difficult to diagnose, it is perhaps well to emphasize at this point that the ordinary case is readily diagnosable and easily differentiated from measles and scarlatina. The exceptional case could be perhaps more easily confused with a drug rash, particularly if the patient had taken a barbiturate during the prodromal period, and there were no history of exposure.

On the other hand, it must be admitted that none of the signs are pathognomonic or always present. Habel produced infection in monkeys from the nasopharyngeal washings of a 4 year old boy in a physician's family, who was definitely exposed to rubella for 3 consecutive days, 17 days

prior to the development of "fever, malaise, and slight rhinitis followed by posterior cervical lymphadenopathy," yet the child never exhibited a rash. Floystrup[16] also reported the "inapparent" infection (*i. e.*, without rash) of his own son, 2 weeks following exposure to rubella. Two weeks later the boy's brother developed the typical picture. The value of accurate knowledge of the incubation period, and the dates of exposure in such circumstances is apparent.

Hynes[25] made leukocyte counts on 37 males, 15 to 39 years old, and on 24 females, 18 to 45, during the first 2 weeks of the disease. He found that a leukopenia was the rule at the onset, with the leukocyte count rising to the upper limit of normal by the tenth day. At the onset, half the patients had an absolute neutropenia, which gradually became corrected. There was also an absolute lymphopenia at the onset, but an absolute lymphocytosis after the fifth day. Türk cells were always present and commonly numerous, and reached their maximum about the fourth day. Plasma cells were present in a half to two-thirds of the patients during the first week. The sedimentation rate was usually normal.

British authors lay much stress on the finding of Türk cells in the blood smear, though it is Diamond's opinion[11] that such findings being non-specific would be of no decisive aid in diagnosis. It is apparent that no adjunct to correct diagnosis should be omitted when rubella occurs during pregnancy. Careful search for possible exposure 18 ± 3 days before the rash, careful examination, prompt consultation before the rash disappears when the history or signs are equivocal, are the obviously indicated procedures. Advances in virology, and serological tests are much needed for aid in diagnosis in such cases.

COMPLICATIONS. *Secondary Infection.* It might be expected that a constitutional virus infection, involving the mucous membranes of the upper respiratory tract, would have secondary infections such as mastoiditis, sinusitis, and the like. Such does not seem to be the fact. Wesselhoeft after long experience with contagious disease believes rubella to differ strikingly from measles in this respect.[44] The National Research Council[34] mentions no such complications among its 1500 reported cases

Secondary infections are, however, occasionally to be reckoned with. Thus in Humphrey's experience[24] with 305 cases occurring among institutionalized children, 5% developed tonsillitis, another 5% developed non-suppurative adenopathy, 1% suppurative adenopathy, and 1% otitis media.

One of us has seen 5 patients in the post-eruptive stage of rubella contract the typical angina and exanthem of scarlet fever. This occurred on a troopship when a patient immune to rubella, suffering from a streptococcal ulcer of the leg, was moved into the sick bay with rubella patients because of lack of space elsewhere. In view of the relative rarity of secondary infection, their variety and the circumstances under which some of them occurred it is a question whether these instances represent actual "complications" or merely coincident infections.

Neuritis. There are rare reports in the literature[21,23] of what has been diagnosed clinically as "neuritis" appearing 3 to 6 days after the appearance of the rash. Pain, numbness or paresthesias of the wrists, shoulders, fingers, hands or ankles, has been complained of by the patient, and in about half the cases transient motor weakness was detected. In 4 such patients where the age is given the average was 32. There is some suggestion that the phenomenon was "arthritic" because of joint involvement and swelling, rather than a true neuritis. The cases were seen in general practice and no one has reported the condition in school epidemics. We have encountered one such case with "rheumatic" puffiness of wrists and ankles for a week following rubella.

Encephalitis. Margolis, Wilson and Top[33] estimated that encephalitis occurs in approximately 1 out of 6000 cases of

rubella. Patients with this complication exhibit the non-specific signs of encephalitis: headache, vomiting, stiff neck, fever, reflex or speech changes, and the like. The authors note that "the leukopenia of rubella becomes a leukocytosis with the onset of encephalitis." Severe and persistent convulsions characterized the course of their 4 fatal cases, and were encountered in 4 of their 10 patients who recovered. The course, whether mild or severe, was found to be short, and death when it occurred (20.8% fatality rate) was within 3 days of the onset of encephalitic signs. These occurred "with striking consistency 4 days after the onset of the disease." They further note that only 1 of the 34 previously reported cases in the literature developed persisting sequelæ —ataxia, of all the extremities, tremor of the right hand and partial facial paralysis. These symptoms cleared in 2 months. One of the authors' cases had similar persisting signs 4 months after onset.

There does not appear to be any special predilection of encephalitis for any one age group. Encephalitis is not mentioned as having been encountered in the more than 1500 cases studied by the British Medical Research Council in boarding schools.

Recrudescences. The great majority of recurrent attacks follow shortly in the wake of the first. Thus in an epidemic reported by Humphrey[24] 19 patients (6%) had recrudescent attacks which occurred from 12 to 41 days after the onset of the first attack. He states "that in our opinion there can be no question concerning the diagnosis. In such instances . . . the child went through the original course of the disease without complication, and then later a second course with similar symptoms, except that in 3 complications developed. . . . The rash was typical and generalized in both of the attacks. It was observed that in general the second attack was of shorter duration and was milder. It should be noted that there was opportunity in the institution for re-exposure." He concluded that "the large number of second attacks suggests that immunity in rubella may not develop for about 3 weeks after onset." The average interval excluding the 1 case with a 41-day intervening period, was 26 days.

In one outbreak of 65 cases brought to our attention, one recrudescent attack occurred following the primary by an interval of 18 days.

Second Attacks. Bona fide second attacks of rubella appear to be rare. That they do occur is suggested by the National Research Council's statement that "in 4 pairs of epidemics analyzed in detail, 2 boys at school N.B. were attacked in both epidemics, the diagnosis being made by the same school medical officer." However, physicians, nurses and parents are continuously being re-exposed without contracting the disease for a second time and the very nature of the epidemiologic curves which are presented here show that immunity is essentially lifelong. It is probable that the recrudescent cases described above account for the widespread impression that rubella is readily contracted for a second time. Misdiagnosed cases and misinformation add to the confusion on this point. Thus the National Research Council finds that "in 9 analyzed epidemics among boys and in 3 among girls, the attack rate on so-called immunes was over 10%." However, the Council doubted that "error can account entirely for this phenomenon."

Inapparent Attacks. There is some evidence that a susceptible individual may acquire immunity to rubella without apparent clinical signs. The National Research Council notes that "in one school, out of 539 boys not attacked in 1932 when the attack rate was 17.1%, 339 were still in school in 1933 when there was a second outbreak. None of these boys was attacked in the second outbreak, whereas 20.4% of the boys exposed for the first time contracted rubella. Perhaps the true explanation lies in the ease with which mild clinical signs may be overlooked.

Rubella—Epidemiology.* In view of the long-held attitude toward German measles as a "nuisance rather than a disease," it is not surprising that little information is to be found which affords much of a conception of the epidemiologic behavior of the disease. Beyond the fact that it is obviously of a contagious nature, data have not been assembled which establish even such general features as the extent of occurrence of the disease, its age distribution, urban-rural behavior, duration of immunity, etc. But on reading the foregoing discussion on incubation period, recrudescences, second attacks and inapparent attacks, one is struck by the similarities to measles. From data presented here, it would be expected that equally complete reporting and study would reveal an epidemiologic pattern in German measles not unlike that of measles.

There are many indications that the reporting of German measles is for apparent reasons highly deficient. Nonetheless the reported occurrence of the disease forms an epidemiologic pattern sufficient to stamp it as one of "the immunizing infections of childhood" of the same magnitude as measles. In Chart 3 is shown the reported incidence of the disease in Massachusetts from 1917 to 1945 by years and by months. There is a fairly regular periodicity differing from measles in that high prevalence tends to extend over periods of 2 or 3 years in sharp contrast with the intervening low prevalence of 2, 3 or 4 years' duration. This is seen in the seasonal curve, similar to that of measles, excepting that during the periods of high prevalence the disease remains at distinctly high levels in the "off season." Not only these general features but the fact that in the highest years the reported cases of German measles attain the same proportion as the highest years of measles is especially suggestive of an extent of spread in German measles similar to that in measles. In lower years the fact that cases run only about one-tenth those of measles is believed to be due to deficiency in reporting.

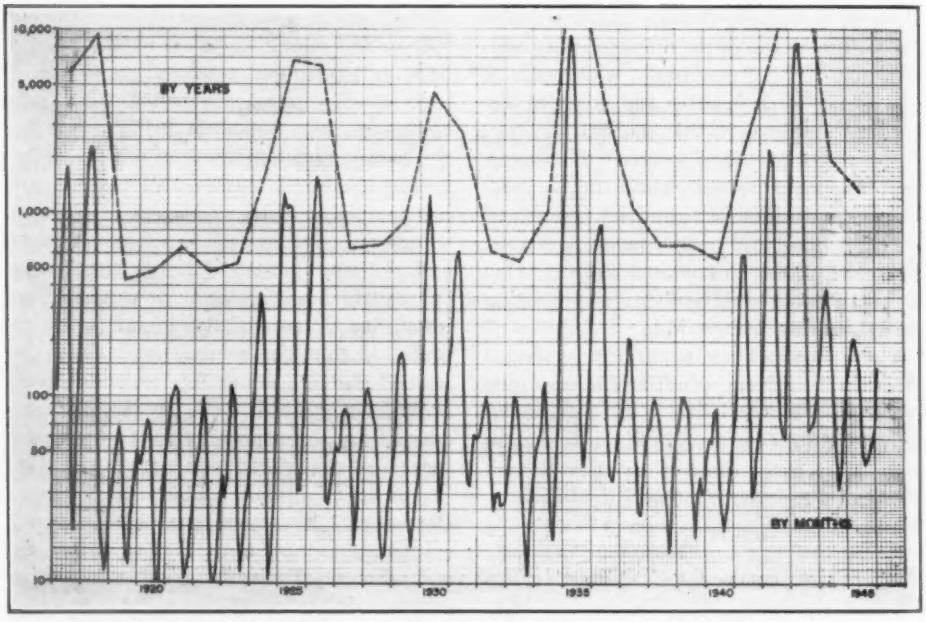

CHART 3.—Reported incidence of rubella by years and by months in Massachusetts.

* We are indebted to the Division of Communicable Diseases, State Department of Health of Massachusetts for epidemiologic data on rubella, and to Miss Katharine H. Hendrie for analysis of it.

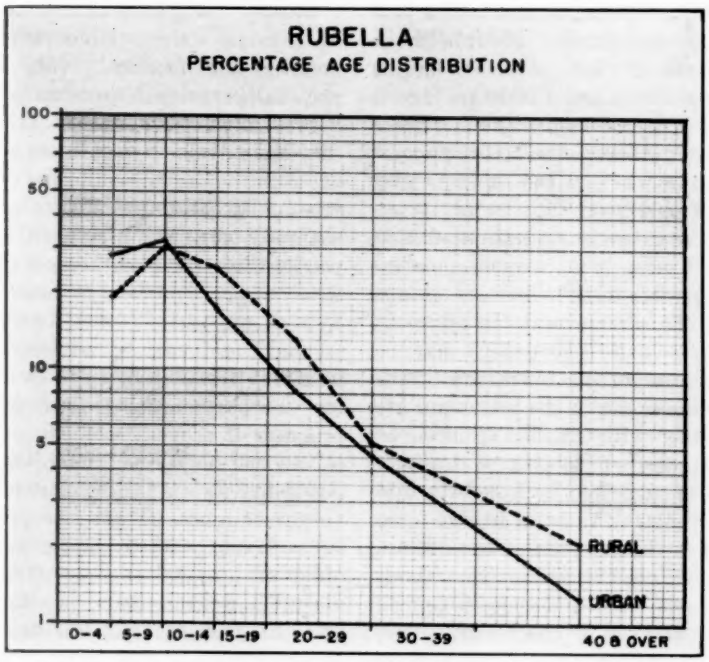

CHART 4.—Comparative age distribution of rubella in urban and rural Massachusetts in 1943.

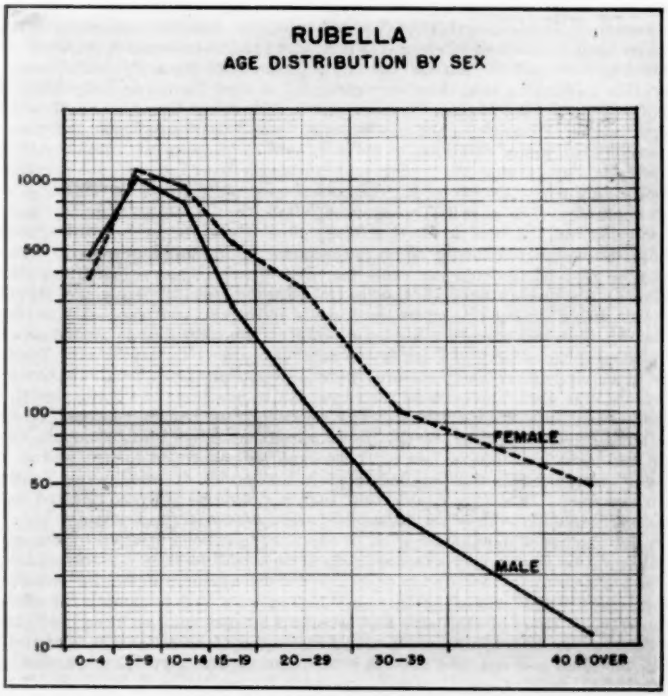

CHART 5.—Reported cases of rubella by age groups and sex in Massachusetts, 1943.

AGE AND SEX DISTRIBUTION. The percentage age distribution of rubella in Massachusetts in 1943 for seven largest cities and for towns under 5000 are shown in Chart 4. The occurrence of the disease at a somewhat older age in smaller populations is characteristic of the "immunizing infections of childhood."

In both the urban and rural population the reported cases in females become increasingly greater in the older age groups (Chart 5). To what extent the excess in females in the older age groups may be due to better reporting by reason of the fact that females are in the home and are reported along with smaller children or to what extent there is greater exposure to children as suggested by Pope's[35] study of secondary attack rates in scarlet fever is not known. In the present connection, the point of interest is the fact that about 14% of female cases and about 8% of all cases are in females of child-bearing age.

Finally it might be stated that there is no seasonal differential between German measles and measles. The curves of seasonal prevalence are closely similar.

Prevention of Anomalies. At the present time the only positive measure is deliberate exposure before the child-bearing period. It would seem undesirable to prevent the naturally occurring infection among young people when their mothers, if susceptible, were not pregnant; and in females actually to seek exposure if they have not contracted the disease by adolescence. It is possible that inoculation* with rubella may be part of the pediatrician's future program of preventive medicine,† just as is vaccination against smallpox. Such a possibility awaits the development of suitable techniques. There is a mortality to be reckoned with:‡ something in the order of 1/30,000 to 1/100,000, but this mortality is to be found in all age groups and the menace that the disease holds

* If it took 100 years to uncover the severe sequelæ of rubella, oddly enough we may have to go back 100 years to uncover a principle applicable to their prevention: namely, inoculation. Variolation, "that most interesting epoch in medical history which kept greater and humbler minds in fever heat during almost one whole century," has entered little, if at all, into later immunologic investigation,[28] although it called forth, in its time, a unique and extensive trial of a specific preventive measure, the logical consequence of which was vaccination. Without attempting to review the activities of many great medical minds of the day, it is interesting that they were extended to such figures as Lady Mary Wortley Montague, Benjamin Franklin, Cotton Mather, Voltaire and Frederick the Great, not to mention the brothers Robert and Daniel Sutton, themselves not medical men, who became specially famous for their "successful inoculation." With many attempts to make it safe by preparation of the patient, such as the cooling regimen of Sydenham so that the "solids and fluids may be reduced from a greater to less inflammability," or modifying the inoculation by dilution and the use of "passage" or "unripe" virus, it might well have furnished an efficacious preventive measure,[28] had it not been quickly supplanted by the safe and practicable vaccination. It was declared a felony in England by an Act of Parliament in 1840.[10] In the manner that the name vaccination came to designate an immunological principle, "variolation" as a principle may be revived (inoculation with the virus of rubella) as constituting the most feasible procedure available at the present time for the control of the disastrous sequelæ of rubella.

Variolation at best was a drastic procedure, and would have been so even if strains like alastrim had been utilized. Its objective was to induce life long immunity by mild strains (based on crude empirical criteria) at the most propitious time and under the most propitious circumstances. Measles is another inevitable disease in which deliberate exposure has been practiced at times, particularly within the family circle. The use of immune globulins permits the physician to postpone the inevitable if the moment is not propitious, or to provoke a milder disease. The possibility of inoculation of infants before passive immunity of maternal origin disappears or the production of modified measles by the combined use of globulin injection and virus inoculation, has not been explored, no doubt for the reason that virus has not been available. A fraction of the population gives no history of ever having contracted rubella, even after known exposure. It is conceivable that such people may have contracted an unrecognized modified form of the disease while they yet possessed partial maternal immunity.

† Incidentally, the practice of inoculation with, or exposure to rubella would provide an unprecedented opportunity for observation on the precise mechanism of natural infection: that aspect of epidemiology which has been least satisfactorily imitated in experimental disease in animals. For example, inoculation or exposure of a group of volunteers is the only satisfactory way of determining the efficacy of gamma globulin as a prophylactic, short of extensive and laborious studies such as have been made on measles during the past decade. A defect inherent in this latter type of investigation is that the differential diagnosis between modified measles and rubella is so tenuous that the differentiation of a failure to immunize from modified measles could occasion considerable confusion.

‡ Figures tending to show that the mortality is 1 to 60,000 may be excessively pessimistic, as it is probable that most cases of post-rubella encephalitis are reported whereas a minority of cases of the uncomplicated disease are thought to be reported.

for the unborn child would be appreciably reduced if not eliminated.

The only prophylactic therapy that can be proffered an exposed pregnant mother is immune globulin. However, available clinical experience has not established its efficacy. There have been a number of instances of evident failure of gamma globulin to prevent the disease. The term "evident failure" is used, since the distinction between modified measles and rubella is not an easy one: rash, Koplik spots, and incubation periods all being altered. In one family, 2 children exposed to both measles and rubella in school developed a fine rash and post-auricular adenopathy 14 and 15 days after an injection (2.5 cc.) of gamma globulin. Of 2 other children in the same family, 1 was given gamma globulin at the time the first cases developed; the other a 6 months old baby was not. Both developed typical rubella 15 days after the first sibling's rash. The mother who had had measles in the past but not rubella also developed typical rubella.[4] There appears to be little reason for believing the disease in the three "failures" was modified measles.

Many authors have gone as far as to suggest that "the justification for therapeutic abortion," if rubella (or other exanthemata) be contracted in the first 2 months of pregnancy, "should be debated." Others have gone so far as to accept the available evidence as sufficient grounds for termination of the pregnancy.

Such a far-reaching question can be approached with wisdom only when there are adequate statistical studies to establish the specific risks of infection at all stages of pregnancy. Knowledge from such studies would have to be interpreted not only in terms of actual risk of congenital anomalies but as well in terms of the "health of the mother" in continuing a pregnancy with such a known risk and finally in terms of an informed public opinion.

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BOOK REVIEWS AND NOTICES

NURSING IN COMMERCE AND INDUSTRY. By BETHEL J. MCGRATH, R.N. Pp. 355. New York: Commonwealth Fund, 1946. Price, $3.00.

WITHOUT defining objectives, the author goes straight to the point indicated in the title and presents a manual, complete, well organized and delightfully written. As such, it fulfills a long felt need of nurses in industry. However, the book far transcends these limits. It holds much of interest to *all* nurses, as well as to executives in industry, to the field of preventive medicine and public health, and to the general public, and also meets the need for a guide to those concerned with the development of health services for students, staff and other personnel in hospitals, schools of nursing and medicine. We note especially the democratic and thought-provoking chapter on relationships with employees and employer organizations. The question of union membership, presented with fairness and restraint, still retains its question mark; the Reviewer would have appreciated stronger support of the professional attitude.

H. F.

AN INTRODUCTION TO ESSENTIAL HYPERTENSION. By RICHARD F. HERNDON, M.D., F.A.C.P. Pp. 88; 7 illus. Springfield, Ill.: Thomas, 1946. Price, $2.50.

THIS little book is clearly written, with frequent summaries and excellent illustrations. It condenses a great deal of material in a relatively small space, and hence should be of value to the physician who wishes to acquire a viewpoint quickly, the physician whom the author describes in the Preface as "the ordinary practitioner on the firing line of medicine." It contains no new or original material, and will probably be of little use to the physician already interested in hypertension. The limitation of space that the author has set for himself necessitates certain omissions that may give the casual reader a distorted view of the outlook for the future.

J. G.

THE VENOUS PULSE AND ITS GRAPHIC RECORDING. By FRANZ M. GROEDEL, M.D., Attending Cardiologist, Beth David Hospital. Pp. 223; 7 ills. and 290 tracings. New York: Brooklyn Medical Press, 1946. Price, $5.50.

THE techniques and interpretations of more than a century of investigation of phlebography, pneumocardiography and esophagocardiography are described and criticized. Technical improvements of methods are demonstrated by many excellent tracing reproductions. An extensive bibliography is provided. This book is difficult reading. Its evaluations are in some cases open to question (*cf.* ballistocardiography). The author is diffuse and repetitive, and seems to overstress the value of these methods in clinical investigation.

C. K.

COSMETICS AND DERMATITIS. By L. SCHWARTZ, M.D., and S. M. PECK, M.D., U. S. Public Health Service. Pp. 189; 20 ills. New York: P. B. Hober, 1946. Price, $4.00.

THE authors concisely present a satisfactory analytical approach to skin reactions to popular cosmetics. The book contains several uninformative photographic illustrations and statements, especially in Chapter I, such as (p. 18): "The hairs which grow in the nostril have the general character of eyelashes. They are only infrequently found in women;" and (p. 19): "Most people have hair on the extremities. In childhood transitional hairs are found. They are later replaced by terminal hairs like those on the body, but shorter;" which tend to decrease rather than increase the reader's interest. Nevertheless, this interest is so amply rewarded in subsequent chapters, that the book is a really valuable contribution to medical literature and should be read and re-read by every physician practising dermatology, as a specialist, or general practitioner.

J. L.

ESSENTIALS OF GENERAL ANESTHESIA. By R. R. MACINTOSH and FREDA B. BANNISTER, University of Oxford. Third ed. Pp. 334; 239 ills. Springfield, Ill.: Charles C Thomas, 1945. Price not given.

IN this third edition, relatively few changes have been made in the text. The book is primarily intended for dentists, but all of the material presented is of interest to the anesthesiologist regardless of the type of surgery contemplated. R. D.

CURRENT THERAPIES OF PERSONALITY DISORDERS. Proceedings of the Thirty-fourth Annual Meeting of the American Psychopathological Association, held in 1945. By BERNARD GLUECK, M.D., Editor. Pp. 300. New York: Grune & Stratton, 1946. Price, $3.50.

THE Presidential Address was entitled: Psychiatry, an Instrument of Personal and Social Rehabilitation. Following this were papers by the various collaborators under these captions: I, The Modern Psychiatric Hospital; II, The Psychochemical Techniques in Psychiatry; III, The Psychiatric Guidance Rehabilitation Techniques.

Group psychotherapy is slowly making headway, so that children, adolescents and adults may be benefited by its application. Relationships with others than their own families are important to children, and group play supplies such a need. Adolescent delinquents may receive benefit. Among adults, the neuropsychoses and mild types of functional psychoses are suitable for group therapy. But not all subjects should receive such treatment; where there are deep-rooted emotional conflicts, perhaps the psychoanalytic method may prove more helpful. Alcoholics Anonymous with its personal, religious and social implications, receives favorable consideration. The employment of convulsive shock therapy in some psychotics, also to prevent deterioration and to maintain certain behavior levels, may prove gratifying when proper amounts are adequately administered, and with careful management of the patients.

Though these contributions are excellent, no book can be all-inclusive. We would have liked to have seen Moreno's psychodramatics considered. N. Y.

NEW BOOKS

Environmental Warmth and Its Measurement. By T. BEDFORD, D.Sc., PH.D., M.I.MIN.E. Pp. 40, Supplement of Charts for the Calculation of Environmental Warmth (H.M.S.O.). London: British Information Services, 1946. Price, $.70.

Women in Industry, Their Health and Efficiency. Issued under the auspices of the Division of Medical Sciences and the Division of Engineering and Industrial Research of the National Research Council. Prepared in the Army Industrial Hygience Laboratory by ANNA M. BAETJER, Sc.D., Assistant Professor of Physiological Hygiene, School of Hygiene and Public Health, The Johns Hopkins Hospital. Pp. 344. Philadelphia: Saunders, 1946. Price, $4.00.

Curare Intocostrin. Composed of reports of one hundred and forty-eight investigators and clinicians published in recent years. Pp. 292. New York: E. R. Squibb & Sons, 1946. No Price Given.

Advancing Fronts in Chemistry. By WENDELL H. POWERS (edited), Assistant Professor of Chemistry. Pp. 156. New York: Reinhold Publishing Corporation, 1946. Price, $3.25.

Medical Service by Government. Local, State, and Federal. By BERNHARD J. STERN, PH.D., Lecturer in Sociology, Columbia University. Pp. 208. New York: The Commonwealth Fund, 1946. Price, $1.50.

New Aspects of John and William Hunter. By JANE M. OPPENHEIMER, Bryn Mawr College. With a Foreword by FENWICK BEEKMAN, M.D. Pp. 208; 5 ills. New York: Henry Schuman, 1946. Price, $6.00.

Wm. Beaumont's Formative Years. With Annotations and an Introductory Essay by GENEVIEVE MILLER, M.A., Institute of the History of Medicine, The Johns Hopkins University. Pp. 87. New York: Henry Schuman, 1946. Price, $6.00.

Squint and Convergence. By N. A. STUTTERHEIM, M.D. (Rand), State Medical Qualification, Holland; formerly Surgeon to the Eye Clinic, University, Leyden; Part-time Ophthalmic Surgeon to the Johannesburg School Clinic, Education Dept., Transvaal. Pp. 95; 26 graphs; 15 diagrams. London: H. K. Lewis & Co., Ltd., 1946. Price, 15/-net ($3.00).

Skin Diseases, Nutrition and Metabolism. By ERICH URBACK, M.D., F.A.C.A., Associate in Dermatology, University of Pennsylvania School of Medicine; Chief of Department of Allergy, Jewish Hospital, Philadelphia. With the collaboration of EDWARD B. LEWINN, B.S., M.D., F.A.C.P., Associate in Medicine, Jewish Hospital, Philadelphia. Pp. 634; 266 ills. New York: Grune & Stratton, Inc., 1946. Price, $10.00.

Diagnostic Examination of the Eye. By CONRAD BERENS, M.D., F.A.C.S., Professor of Clinical Ophthalmology, Columbia University; Executive Eye Surgeon, New York Eye and Ear Infirmary; formerly Chairman, Section on Ophthalmology of the American Medical Association; Managing Director, The Ophthalmological Foundation, Inc.; President, Snyder Ophthalmic Foundation; formerly Chairman, American Board of Ophthalmology; Consultant to the Air Surgeon of the Army Air Forces; and JOSHUA ZUCKERMAN, B.Sc., M.D., C.M., F.A.C.S., Instructor in Ophthalmology, New York University; Instructor in Ophthalmology, New York Eye and Ear Infirmary and Columbia University Postgraduate School; Ophthalmic Surgeon, Midtown Hospital; Assistant Ophthalmic Surgeon, New York Eye and Ear Infirmary; Diplomate, American Board of Ophthalmology; Fellow, American Academy of Ophthalmology and Otolaryngology. Pp. 711; 410 ills. Philadelphia: J. B. Lippincott Company, 1946. Price, $15.00.

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Medical Biochemistry. By MARK R. EVERETT, PH.D., Professor of Biochemistry, University of Oklahoma School of Medicine. Pp. 767; 106 tables. New York: Paul B. Hoeber, Inc., 1946. Price, $7.00.

The Modern Treatment of Diabetes Mellitus. By WILLIAM S. COLLENS, B.S., M.D., Chief of the Diabetic Clinic; Chief of the Clinic for Peripheral Vascular Diseases and Associate Visiting Physician, Israel Zion Hospital, Brooklyn; Associate Visiting Physician, Greenpoint Hospital, Brooklyn; Attending Metabolist, Jewish Sanitarium and Hospital for Chronic Diseases, Brooklyn; Consultant in Metabolic Diseases, Rockaway Beach Hospital, New York. Pp. 514; 195 ills. Springfield: Charles C Thomas, Publisher, 1946. Price, $8.50.

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Peptic Ulcer. By I. W. HELD, M.D., F.A.C.P., Attending Physician, Beth Israel Hospital; Clinical Professor of Medicine (Retired); New York University, College of Medicine, New York City; and A. ALLEN GOLDBLOOM, M.D., F.A.C.P., Assistant Clinical Professor of Medicine, New York Medical College and Flower-Fifth Avenue Hospital; Associate Physician, Beth Israel Hospital and Metropolitan Hospitals; Associate Cardiologist, Beth Israel Hospital, New York City. Pp. 382; 110 ills. Springfield: Charles C Thomas, Publisher, 1946. Price, $6.50.

A History of Medicine. By DOUGLAS GUTHRIE, M.D., F.R.C.S.ED., F.R.S.E. With an Introduction by SAMUEL C. HARVEY, M.D., F.A.C.S., Wm. H. Carmalt Professor of Surgery, Yale University School of Medicine. Pp. 449; 72 ills. Philadelphia: J. B. Lippincott Company, 1946. Price, $6.00.

Psychotherapy in General Medicine. By GEDDES SMITH, Associate, The Commonwealth Fund. Pp. 38. New York: The Commonwealth Fund, 1946. Price, $.25.

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The Diagnosis and Treatment of Pulmonary Tuberculosis. By MOSES J. STONE, M.D., Assistant Professor of Medicine, Boston University School of Medicine; Instructor in Medicine, Tufts Medical School; Physician-in-Chief, Chest Clinics, Beth Israel Hospital and Massachusetts Memorial Hospitals, Boston; and PAUL DUFAULT, M.D., F.A.C.P., Superintendent of the Rutland State Sanatorium, Rutland, Mass. Pp. 325; 93 engr. Philadelphia: Lea & Febiger, 1946. Price, $3.50.

Pneumoperitoneum Treatment. By ANDREW LADISLAUS BANYAI, M.D., F.A.C.P., F.C.C.P., Associate Clinical Professor of Medicine, Marquette University Medical School, Milwaukee, Wis.; Member, Editorial Board, "Diseases of the Chest;" formerly Preceptor in Tuberculosis, School of Medicine, University of Wisconsin, Madison, Wis. Pp. 375; 78 ills. St. Louis: The C. V. Mosby Company, 1946. Price, $6.50.

Ocular Muscles and Fusion. By THOMAS G. ATKINSON, M.D. Pp. 192. Chicago: The Professional Press, Inc., 1946. Price, $3.50.

Studies in Hypertony and the Prevention of Disease. By I. HARRIS, M.D., Honorary Director, Institute for Prevention of Disease; Honorary Physician, Liverpool Heart Hospital. In Coöperation with J. T. IRELAND, B.Sc. (Hons.), A.I.C., Leverhulme Research Fellow; G. V. JAMES, M.Sc., A.I.C., Maurice Stern Research Fellow; EDWARD CRONIN LOWE, M.B.E., M.B., B.S., Director, Pathological Dept., Southport Infirmary; Honorary Clinical Pathologist, Institute for Prevention of Disease; C. E. VERNON, M.Sc., A.I.C., Research Fellow. Pp. 114. Baltimore: The Williams & Wilkins Company, 1946. Price, $3.00.

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Proceedings of the Conference on Diagnosis in Sterility. Edited by EARL T. ENGLE, Chairman, Committee on Research, National Committee of Maternal Health. Pp. 237. Springfield: Charles C. Thomas, 1946. Price, $5.00.

The American Hospital. By E. G. L. CORWIN, PH.D., Executive Secretary, Committee on Public Health Relations, The New York Academy of Medicine; Honorary Charter Fellow of the American College of Hospital Administrators; Former Secretary General and Honorary President of The International Hospital Association. Pp. 226. New York: The Commonwealth Fund, 1946. Price, $1.50.

Peripheral Vascular Diseases. By EDGAR V. ALLEN, B.S., M.A., M.D., M.S. in Medicine, F.A.C.P., Division of Medicine, Mayo Clinic; Associate Professor of Medicine, Mayo Foundation, Graduate School, University of Minnesota; Diplomate of the American Board of Internal Medicine; NELSON W. BARKER, B.A., M.D., M.S. in Medicine, F.A.C.P. Division of Medicine, Mayo Clinic, Associate Professor of Medicine, Mayo Foundation, Graduate School, University of Minnesota; Diplomate of the American Board of Internal Medicine; EDGAR A. HINES, JR., M.D., B.S., M.A., M.S. in Medicine, F.A.C.P. Division of Medicine, Mayo Foundation Graduate School, University of Minnesota with Associates in the Mayo Clinic and Mayo Foundation. Pp. 871; 386 ills. Philadelphia: W. B. Saunders Company, 1946. Price, $10.00.

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Electrocardiography in Practice. By ASHTON GRAYBIEL, M.D., Captain, Medical Corps, U. S. Naval Reserve Coördinator of Research, U. S. Naval School of Aviation Medicine, Pensacola, Fla.; and PAUL D. WHITE, M.D., Lecturer in Medicine, Harvard Medical School; Physician, Massachusetts General Hospital. With the Assistance of LOUISE WHEELER, A.M., Executive Secretary, The Cardiac Laboratory, Massachusetts General Hospital; CONGER WILLIAMS, M.D., Assistant in Medicine, Harvard Medical School and Massachusetts General Hospital. 2nd ed. Pp. 458; 323 ills. Philadelphia: W. B. Saunders Company, 1946. Price, $7.00.

The Surgical Technic of Abdominal Operations. By JULIUS L. SPIVACK, M.D., LL.D., Associate Professor of Surgery, University of Illinois College of Medicine; Diplomate American Board of Surgery; Corresponding Member Mexican Academy of Surgery; Honorary Member, Medical Chapter, Mexican Red Cross; Senori Attending Surgeon, Columbus Memorial Hospital, Chicago; Attending Surgeon, Oak Forest Infirmary. 4th ed. Pp. 712, 682 ills. Springfield: Charles C Thomas, 1946. Price, $10.00.

The Management of Fractures, Dislocations and Sprains. By JOHN ALBERT KEY, B.S., M.D., St. Louis, Mo.; Clinical Professor of Orthopedic Surgery, Washington University School of Medicine; Associate Surgeon, Barnes, Children's and Jewish Hospitals; and H. EARLE CONWELL, M.D., F.A.C.S., Birmingham, Ala.; Orthopedic Surgeon to the Tennessee Coal, Iron and Railroad Company and the American Cast Iron Pipe Company; Chairman of the Committee on Fractures and Traumatic Surgery of the American Academy of Orthopedic Surgeons; Member of the Fracture Committee of the American College of Surgeons; Associate Surgical Director of the Crippled Children's Hospital; Attending Orthopedic Surgeon to St. Vincent's Hospital, South Highlands Hospital, Jefferson-Hillman Hospital, Children's Hospital, Jefferson-Hillman Hospital, Children's Hospital and Baptist Hospitals, Birmingham, Ala. 4th ed. Pp. 1322; 1318 ills. St. Louis: The C. V. Mosby Company, 1946. Price, $12.50.

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Textbook of Biochemistry. By BENJAMIN HARROW, PH.D., Professor of Chemistry, City College, College of the City of New York. 4th ed. Pp. 592; 144 ills. Philadelphia: W. B. Saunders Company, 1946. Price, $4.25.

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Physiological Chemistry. By J. F. MCCLENDON, PH.D., Research Professor of Physiology, Hahnemann Medical College, Philadelphia; formerly Professor of Physiological Chemistry, Medical School, University of Minnesota, Minneapolis. 7th ed. Pp. 463; 83 ills. St. Louis: The C. V. Mosby Company, 1946. Price, $4.25.

A Manual of Tuberculosis. By E. ASHWORTH UNDERWOOD, M.A., B.Sc., M.D., D.P.H., Medical Officer of Health and Chief Administrative Tuberculosis Officer, County Borough of West Ham; Lecturer in the Royal College of Nursing; Fellow of the Royal Statistical Society. With an Introduction by PROFESSOR J. R. CURRIE, M.A., M.D., F.R.C.P., D.P.H., Emeritus Professor of Public Health in the University of Glasgow. 3rd ed. Pp. 524. Baltimore: The Williams & Wilkins Company, 1945. Price, $4.50.

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